Autophagy and Diseases
(Cancer)
The Role of GABARAP Gene in Cancer

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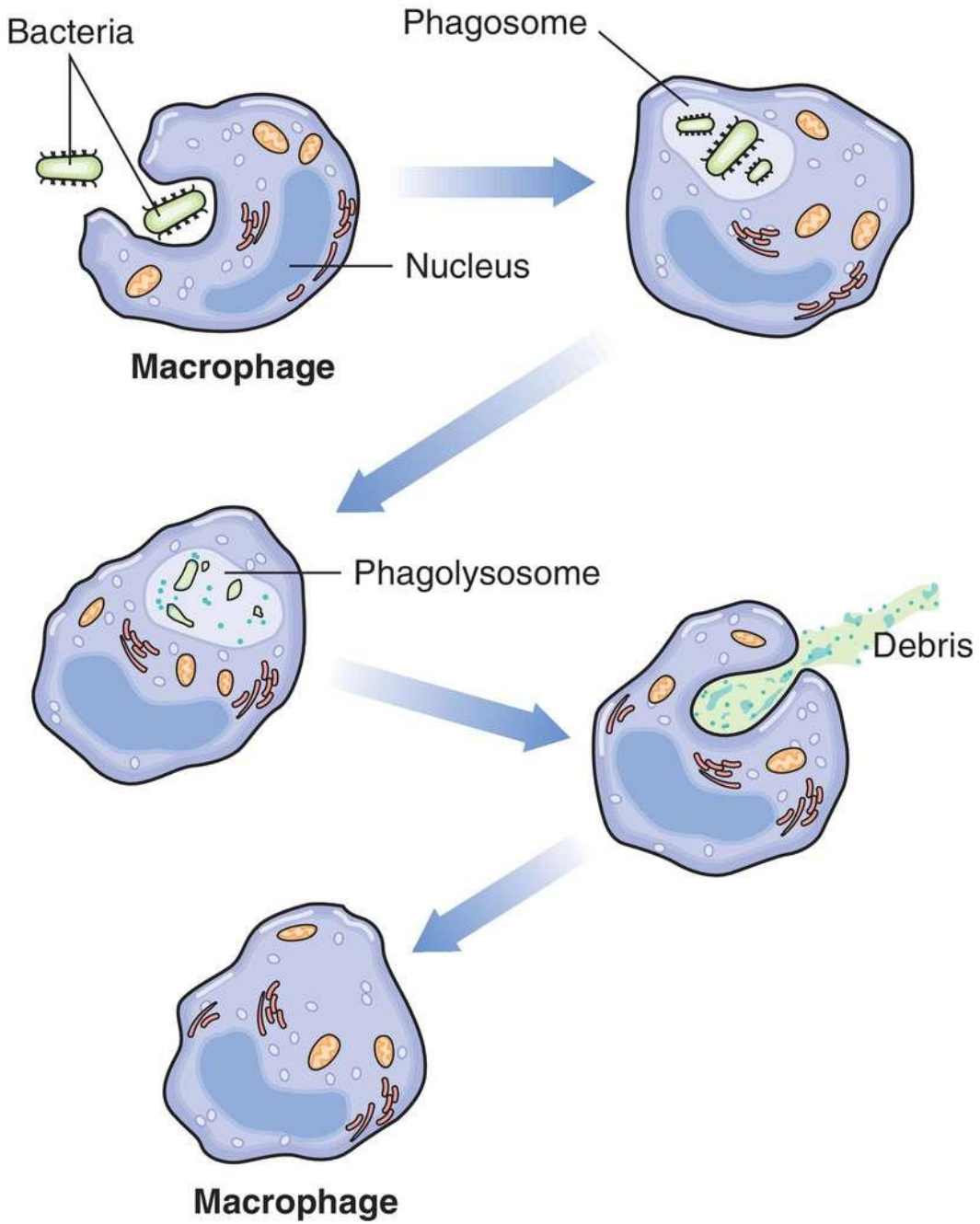
1. Dec. 2016

Phagocytosis



What is Autophagy?

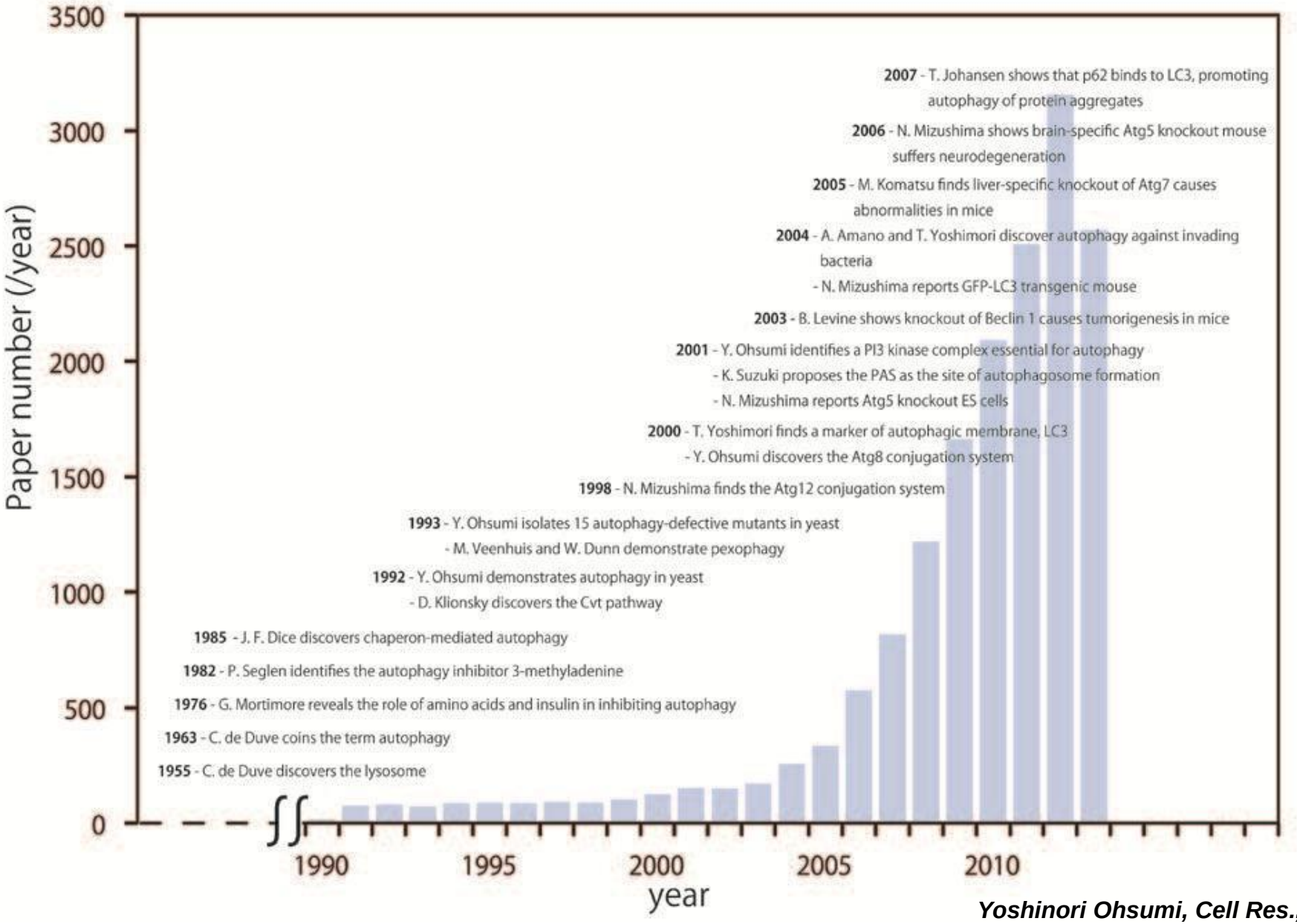
“Self-eating”

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

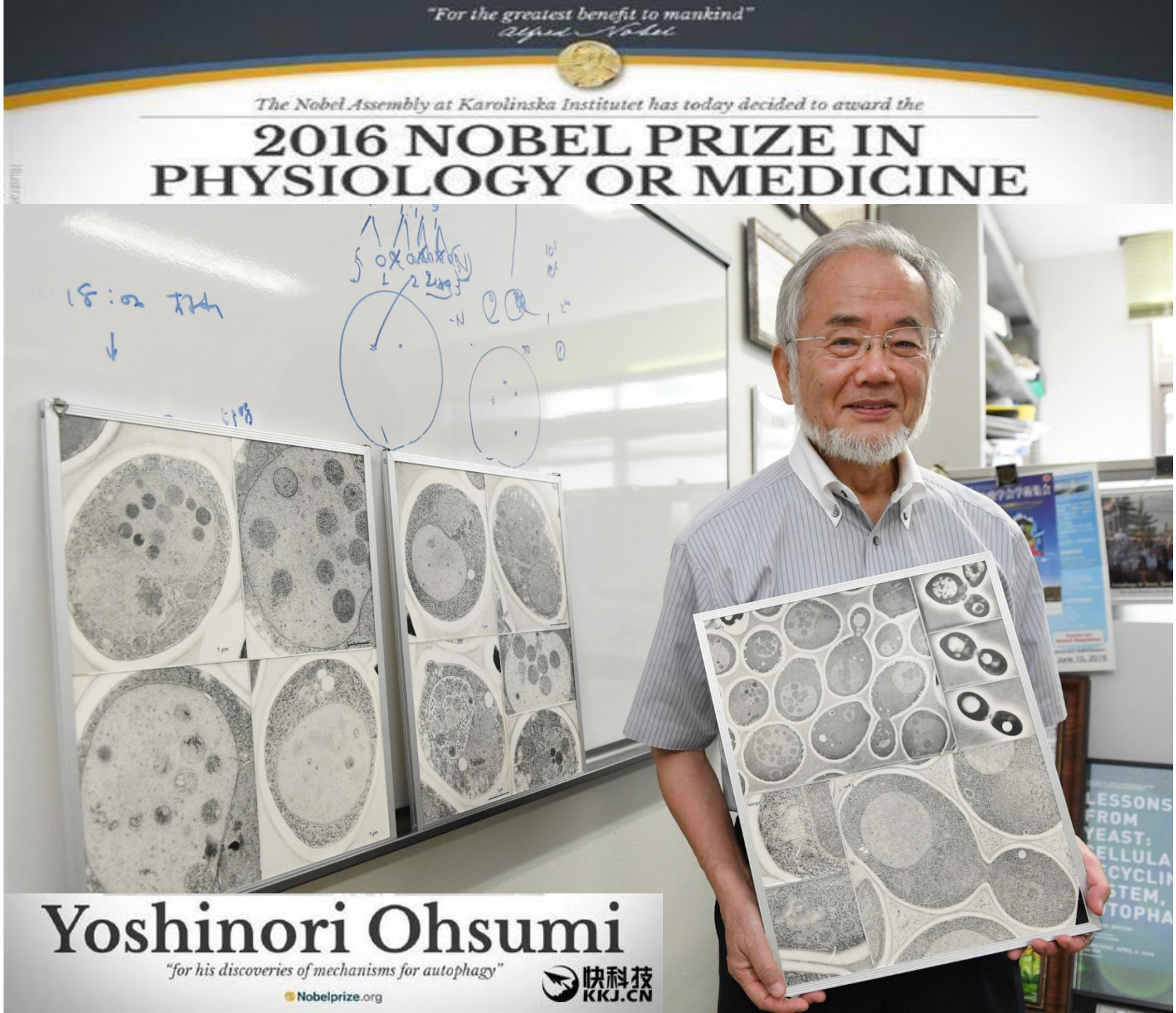
Catabolic process through which the cell recycles its own constituents.

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

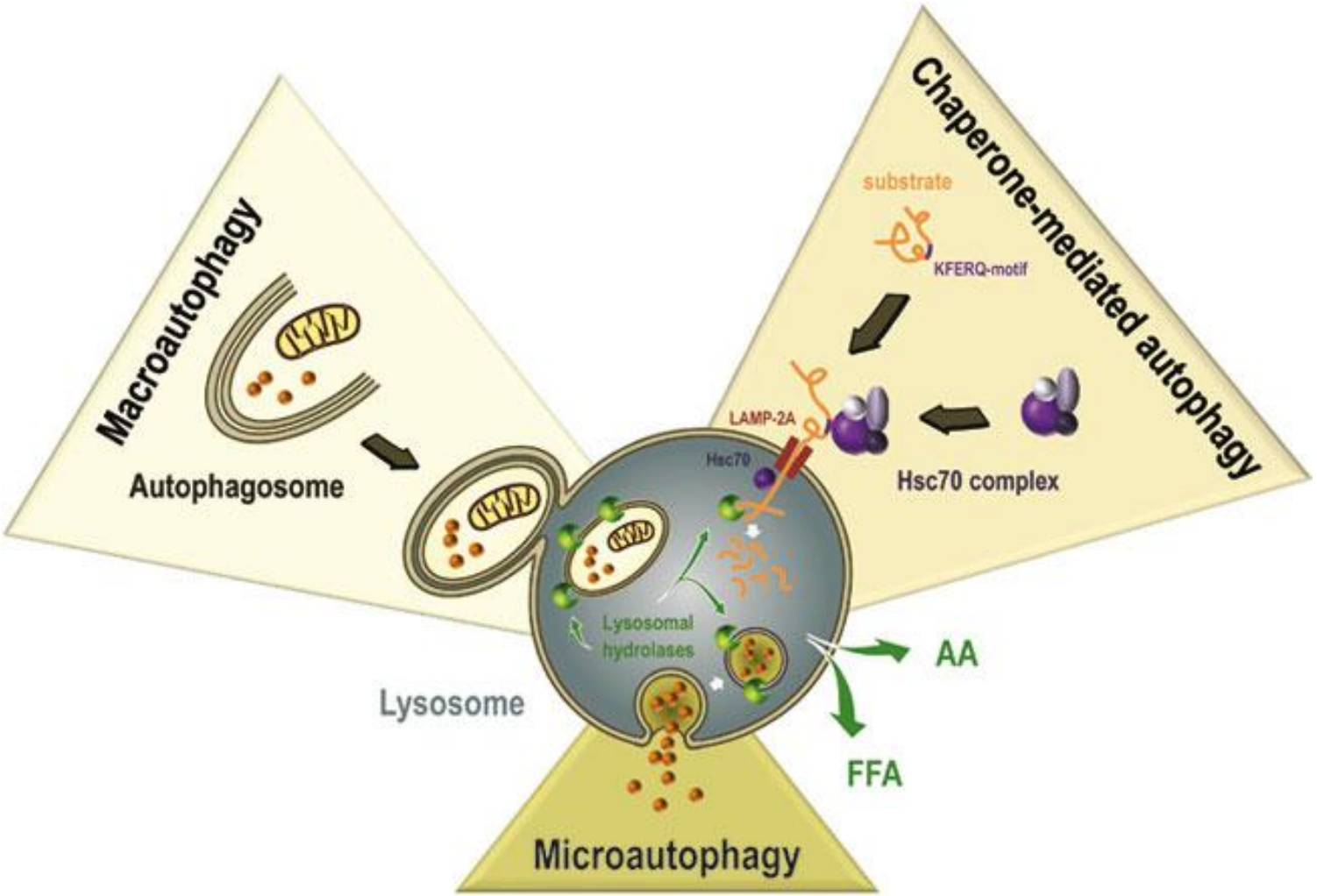
Chronology



Yoshinori Ohsumi, Cell Res., 2014


"For the greatest benefit to mankind"
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

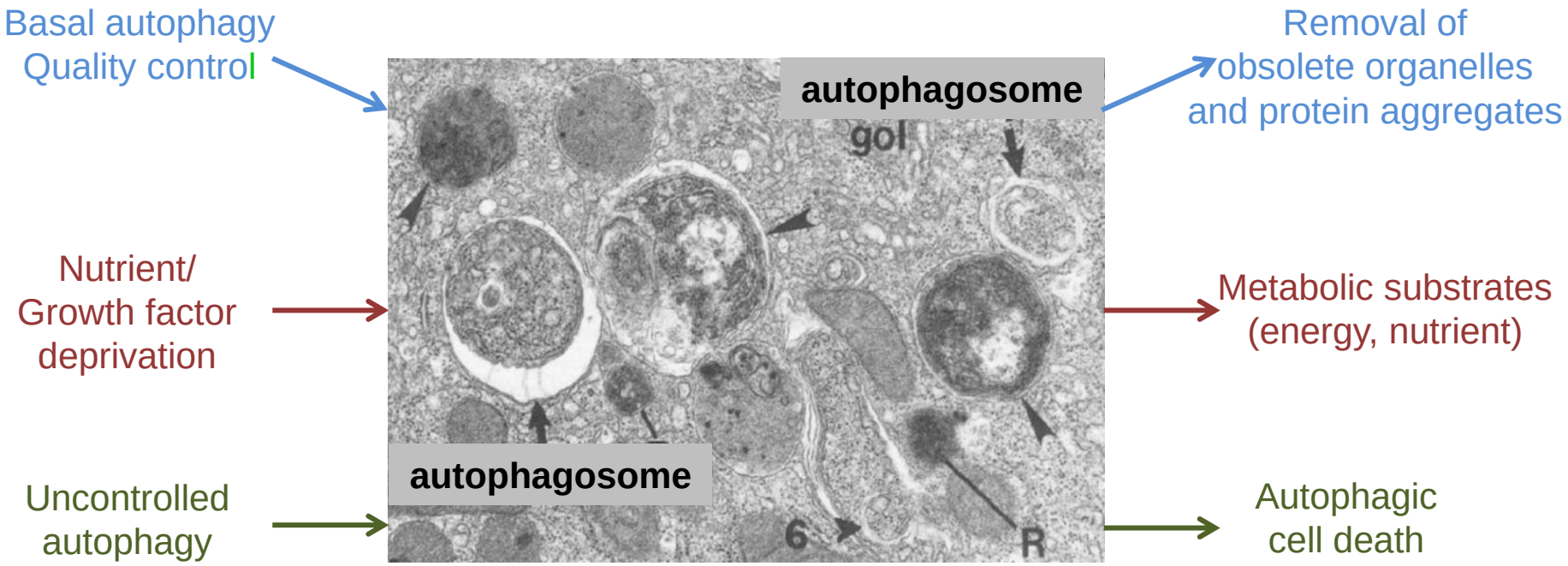
Types of Autophagy



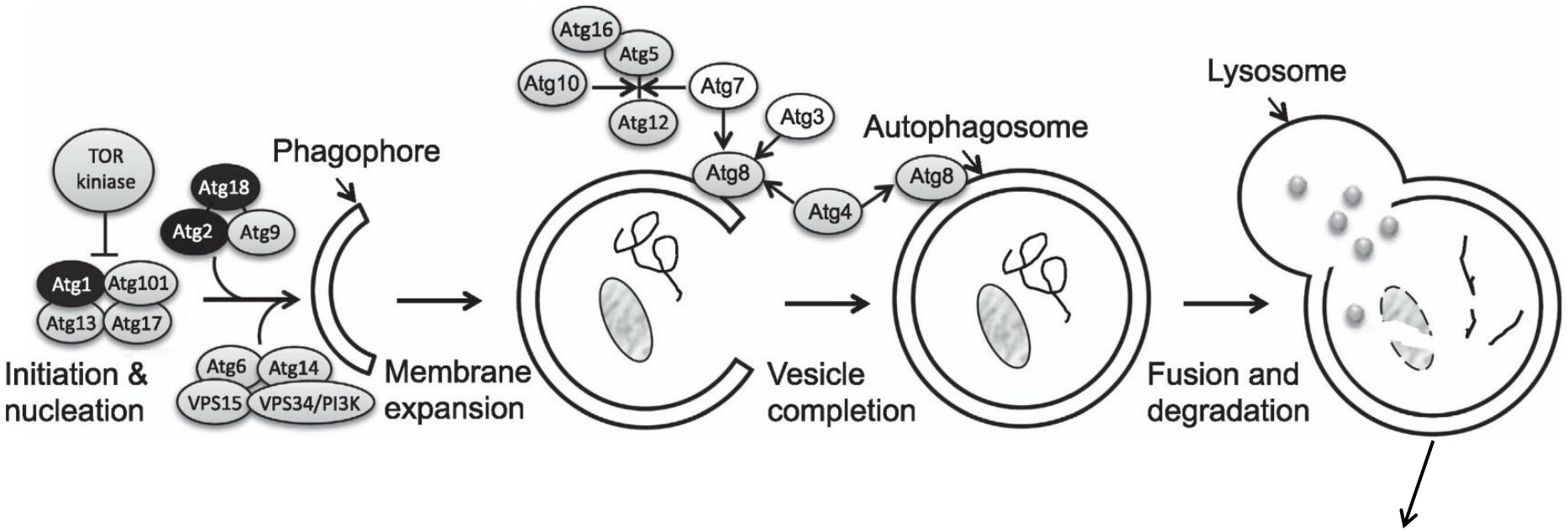
Wirawan et al., Cell Res., 2012

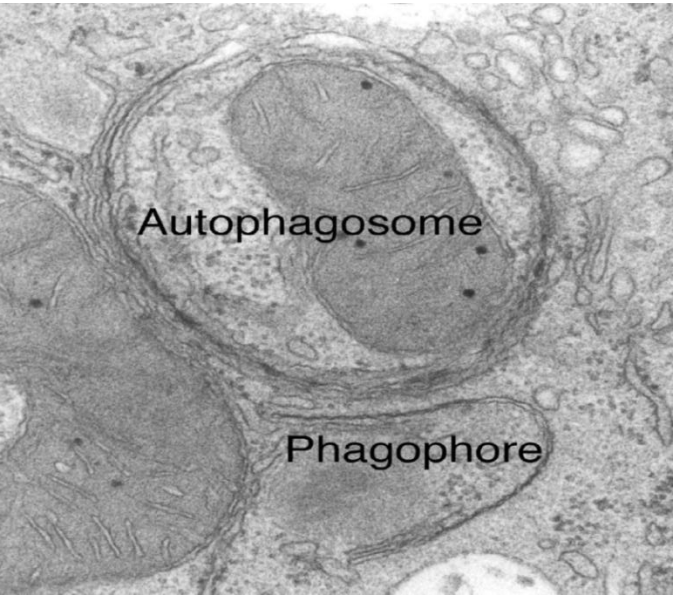
Multiple Functions of Autophagy

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



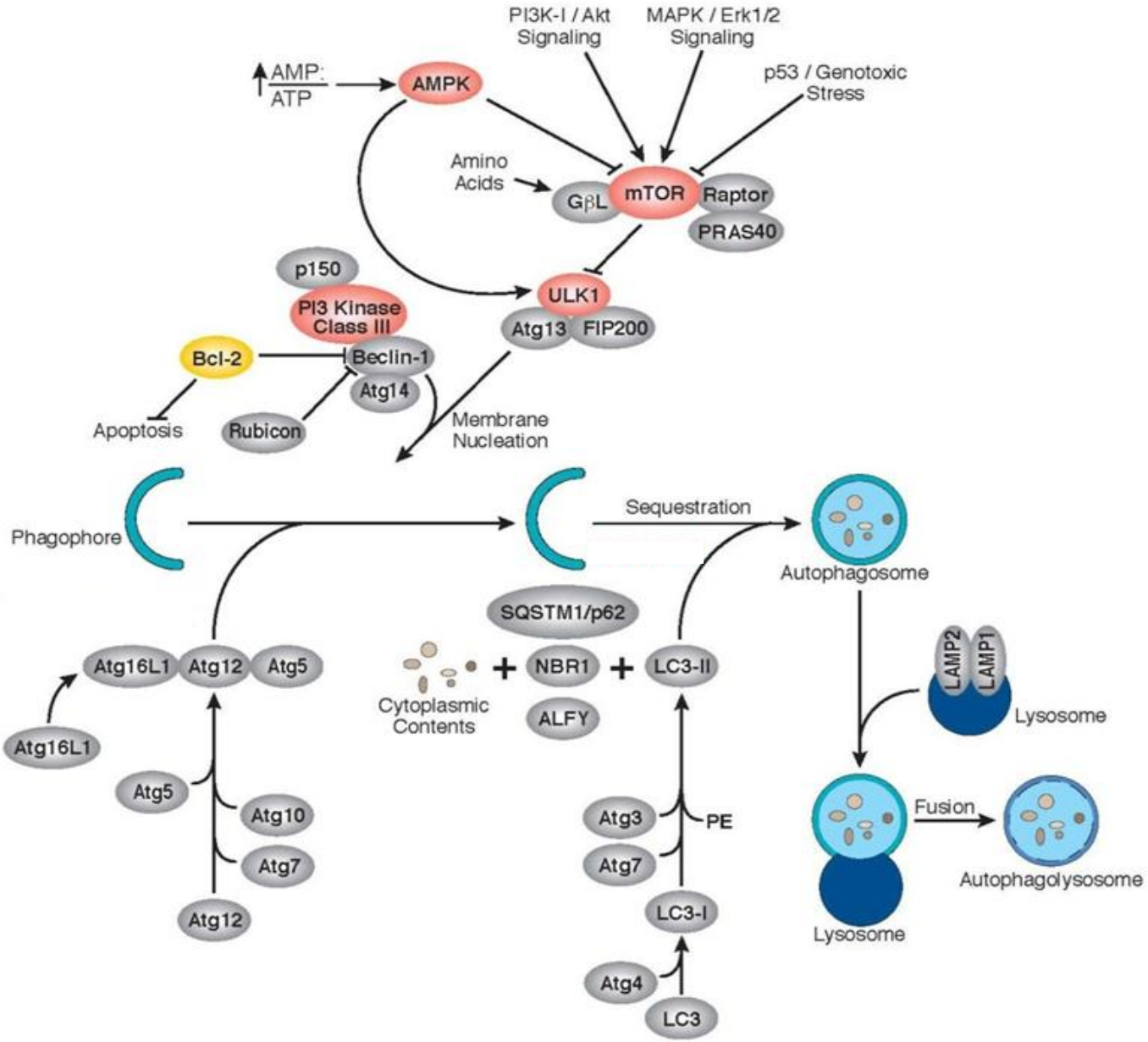
Mechanism of Autophagy



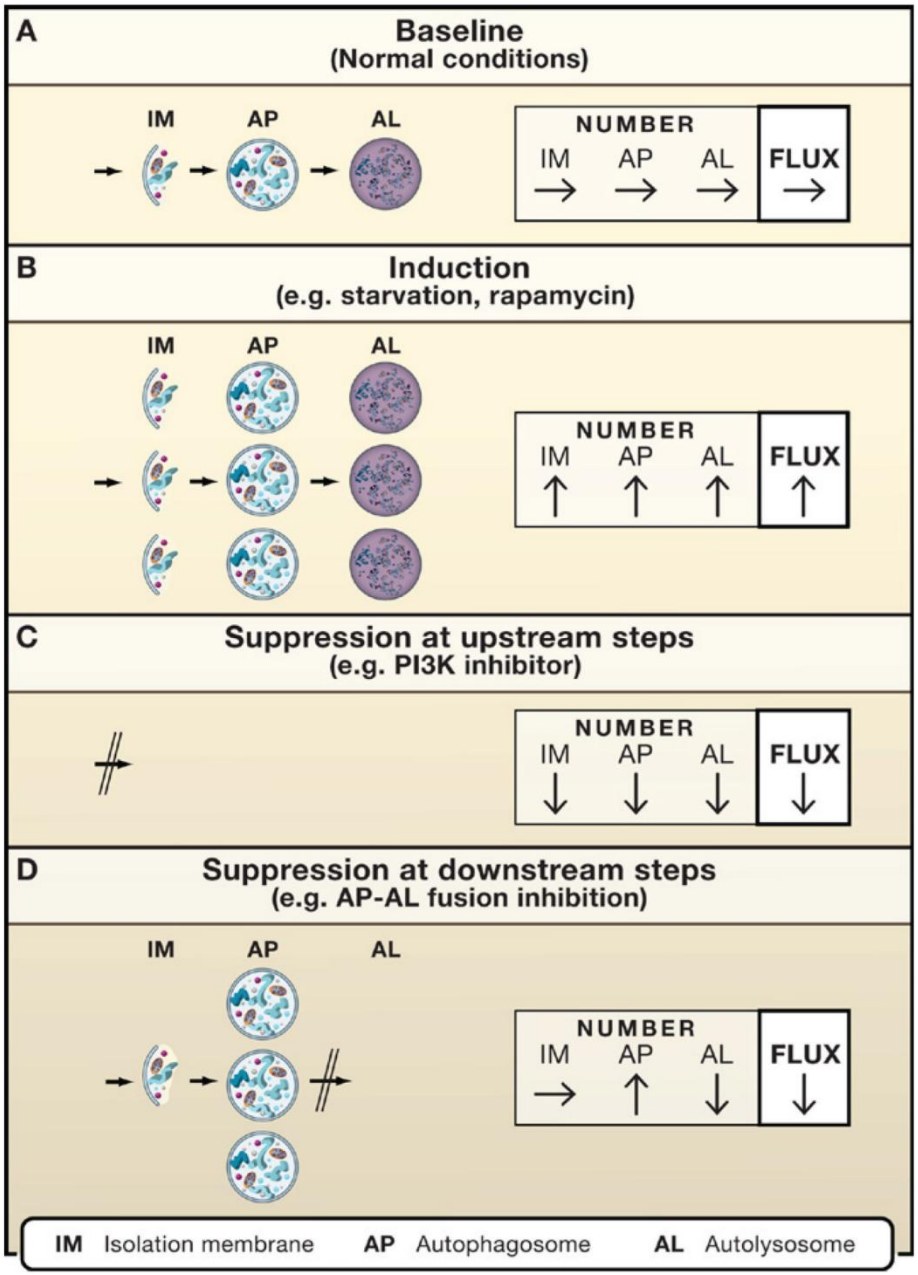


Denton et al., Immunol Cell Biol., 2015

Autophagy Signalling Pathway



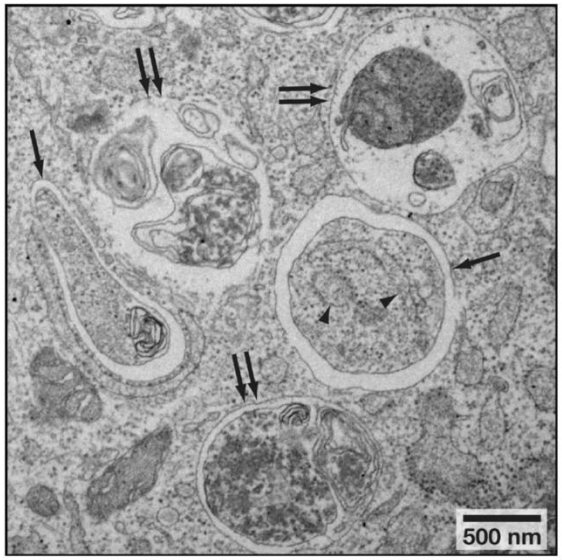
Dynamic regulation of autophagy



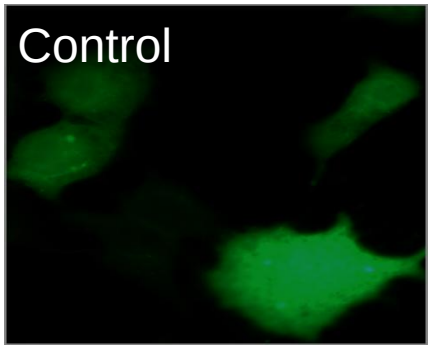
Mizushima et al, Cell, 2010

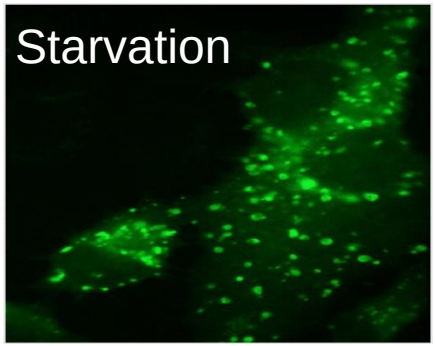
How can We Monitor Autophagy?

EM



IF LC3





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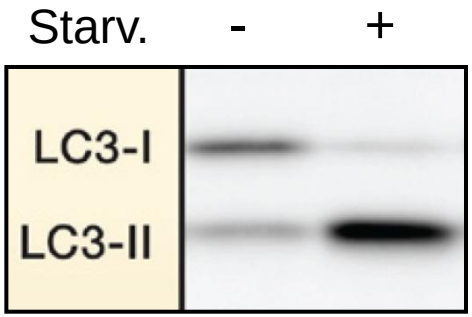

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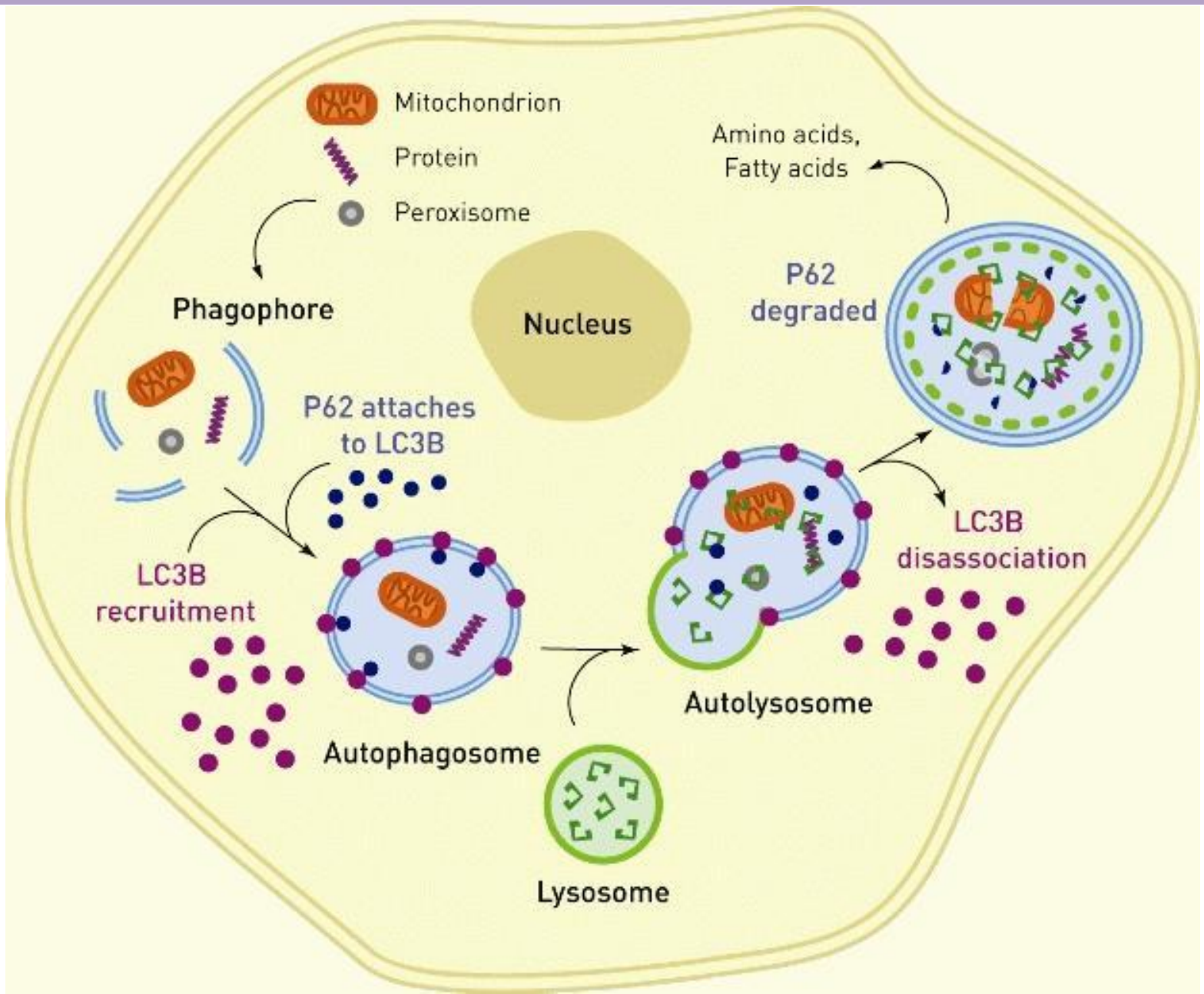
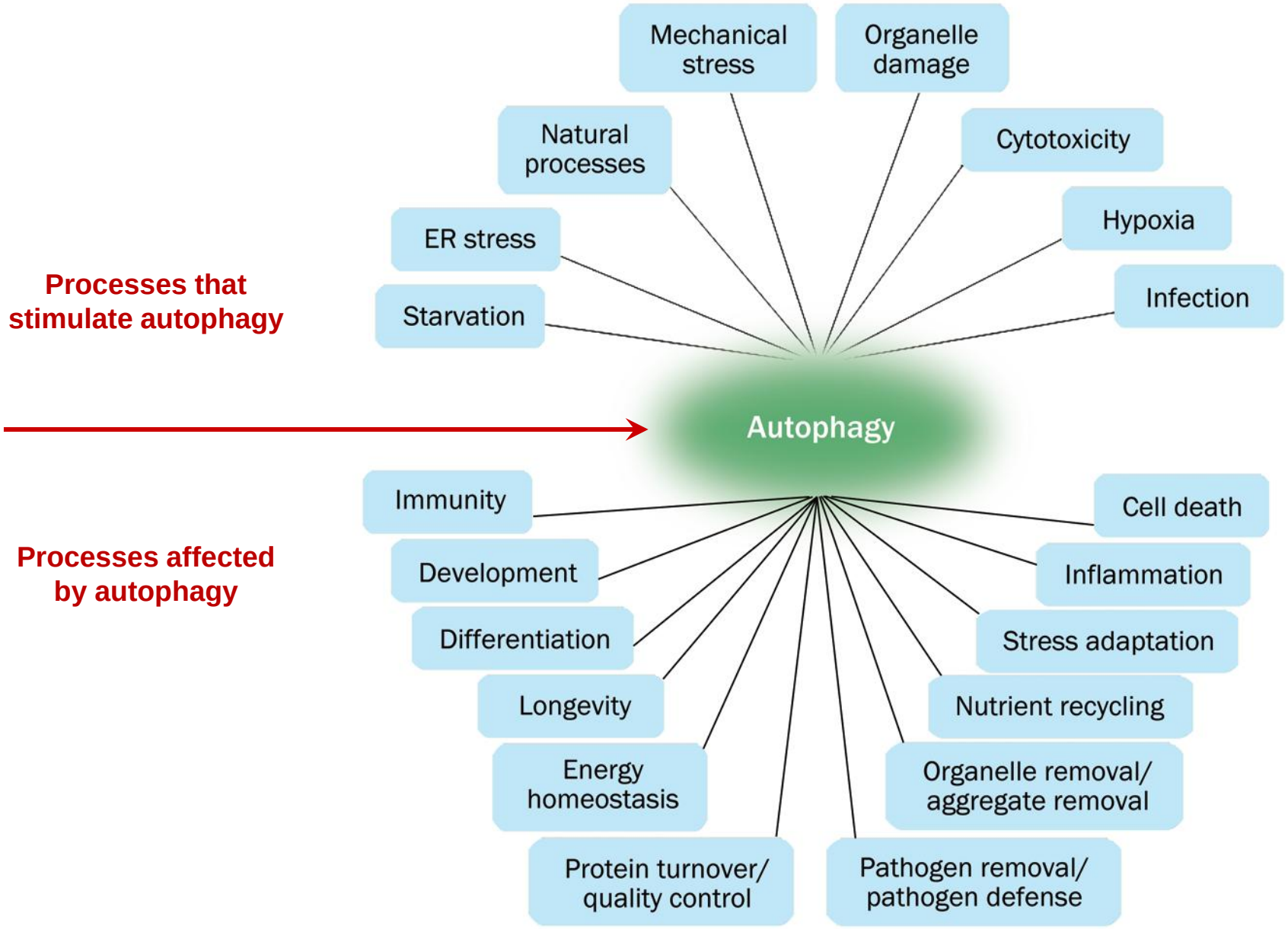


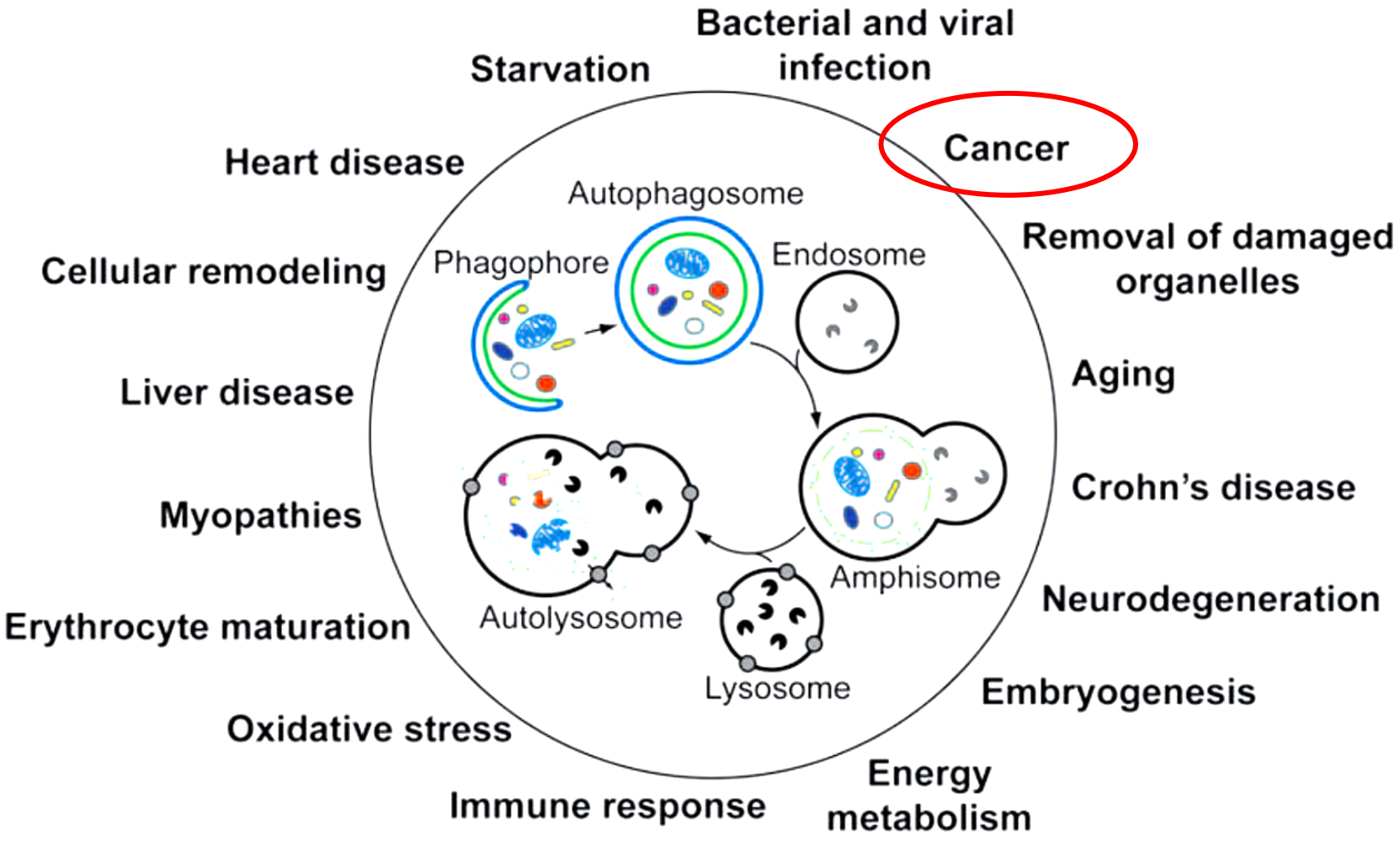
Induction of Autophagy

Processes that stimulate autophagy



Processes affected by autophagy

Autophagy and Diseases



Klionsky DJ, Dev Cell. 2010

Autophagy and Cancer

The connections between autophagy and cancer occur at two aspects:

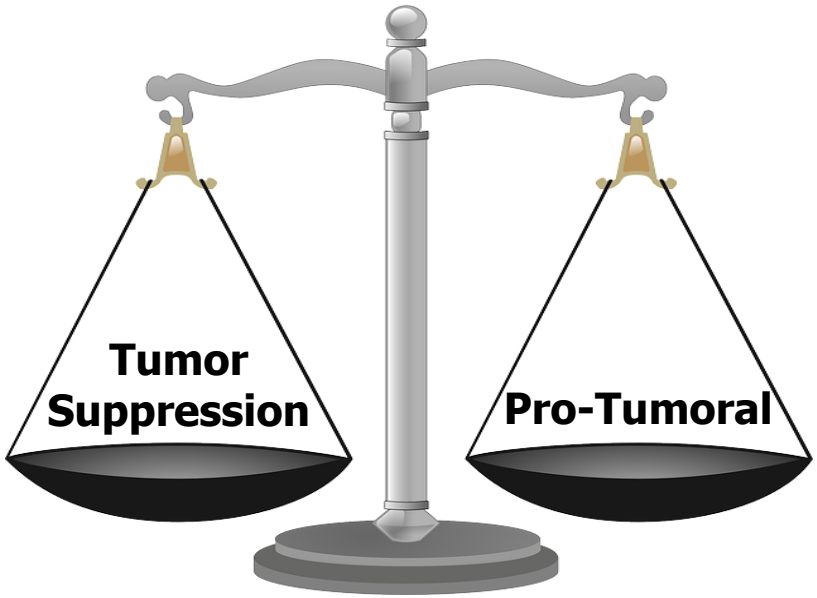
First at the level of tumor initiation and progression,

Second during cancer treatment.

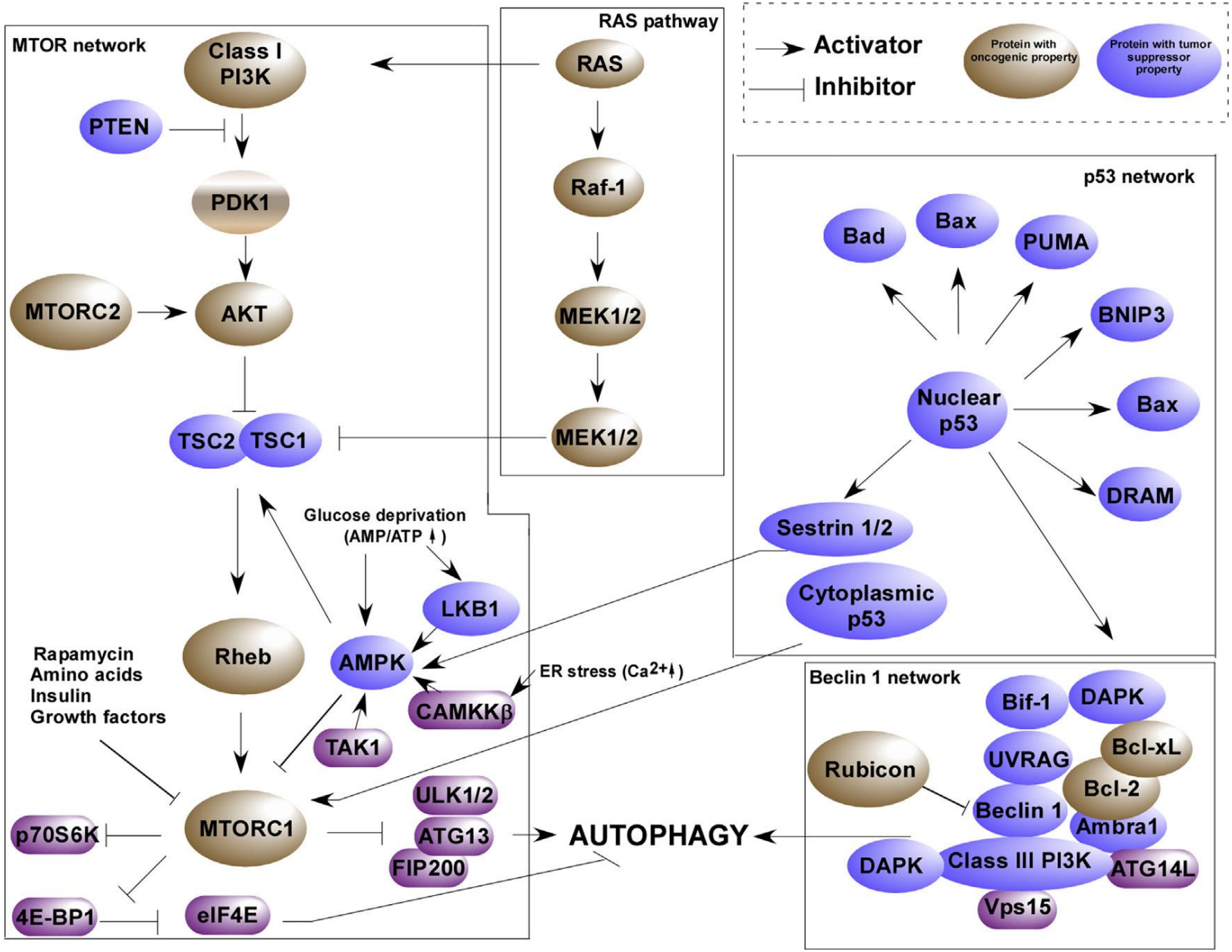
Autophagy in Tumor Initiation and Progression

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

Dual role of Autophagy



Autophagy in Tumor Initiation and Progression



Lorin et al., Seminars in Cancer Biology, 2013

Autophagy and Cancer

❖ **Mouse models for autophagy-deficient gene:**

- Beclin1: tumor suppression function
- ATG5, ATG7, and FIP200: No malignant tumor development *in vivo*.
- GABARAP: less tumor formation after carcinogen treatment

Cell Death Dis. 2016 Apr 28;7:e2205. doi: 10.1038/cddis.2016.93.

Tumor suppression in mice lacking GABARAP, an Atg8/LC3 family member implicated in autophagy, is associated with alterations in cytokine secretion and cell death.

Salah FS[1,2], Ebbinghaus M[3], Muley VY[4,5], Zhou Z[6], Al-Saadi KR[2], Pacyna-Gengelbach M[7], O'Sullivan GA[8], Betz H[8,9], König R[4,5], Wang ZQ[8,10], Bräuer R[1], Petersen I[1].

Author information

Abstract

GABARAP belongs to an evolutionary highly conserved gene family that has a fundamental role in autophagy. There is ample evidence for a crosstalk between autophagy and apoptosis as well as the immune response. However, the molecular details for these interactions are not fully characterized. Here, we report that the ablation of murine GABARAP, a member of the Atg8/LC3 family that is central to autophagosome formation, suppresses the incidence of tumor formation mediated by the carcinogen DMBA and results in an enhancement of the immune response through increased secretion of IL-1β, IL-6, IL-2 and IFN-γ from stimulated macrophages and lymphocytes. In contrast, TGF-β1 was significantly reduced in the serum of these knockout mice. Further, DMBA treatment of these GABARAP knockout mice reduced the cellularity of the spleen and the growth of mammary glands through the induction of apoptosis. Gene expression profiling of mammary glands revealed significantly elevated levels of Xaf1, an apoptotic inducer and tumor-suppressor gene, in knockout mice. Furthermore, DMBA treatment triggered the upregulation of pro-apoptotic (Bid, Apaf1, Bax), cell death (Tnfrsf10b, Ripk1) and cell cycle inhibitor (Cdkn1a, Cdkn2c) genes in the mammary glands. Finally, tumor growth of B16 melanoma cells after subcutaneous inoculation was inhibited in GABARAP-deficient mice. Together, these data provide strong evidence for the involvement of GABARAP in tumorigenesis in vivo by delaying cell death and its associated immune-related response.

PMID: 27124579 PMCID: PMC4855672 DOI: 10.1038/cddis.2016.93

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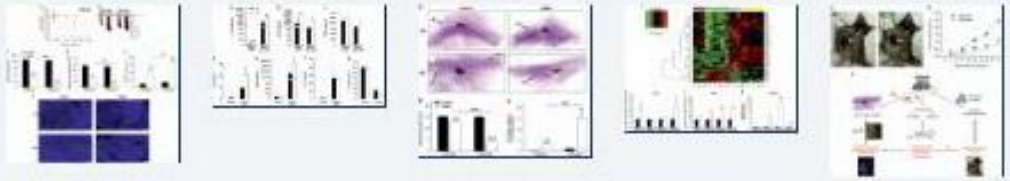
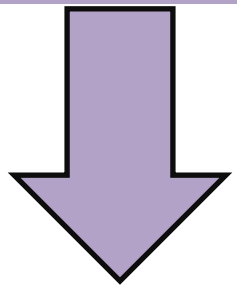


Autophagy as Tumor Suppressor Mechanism

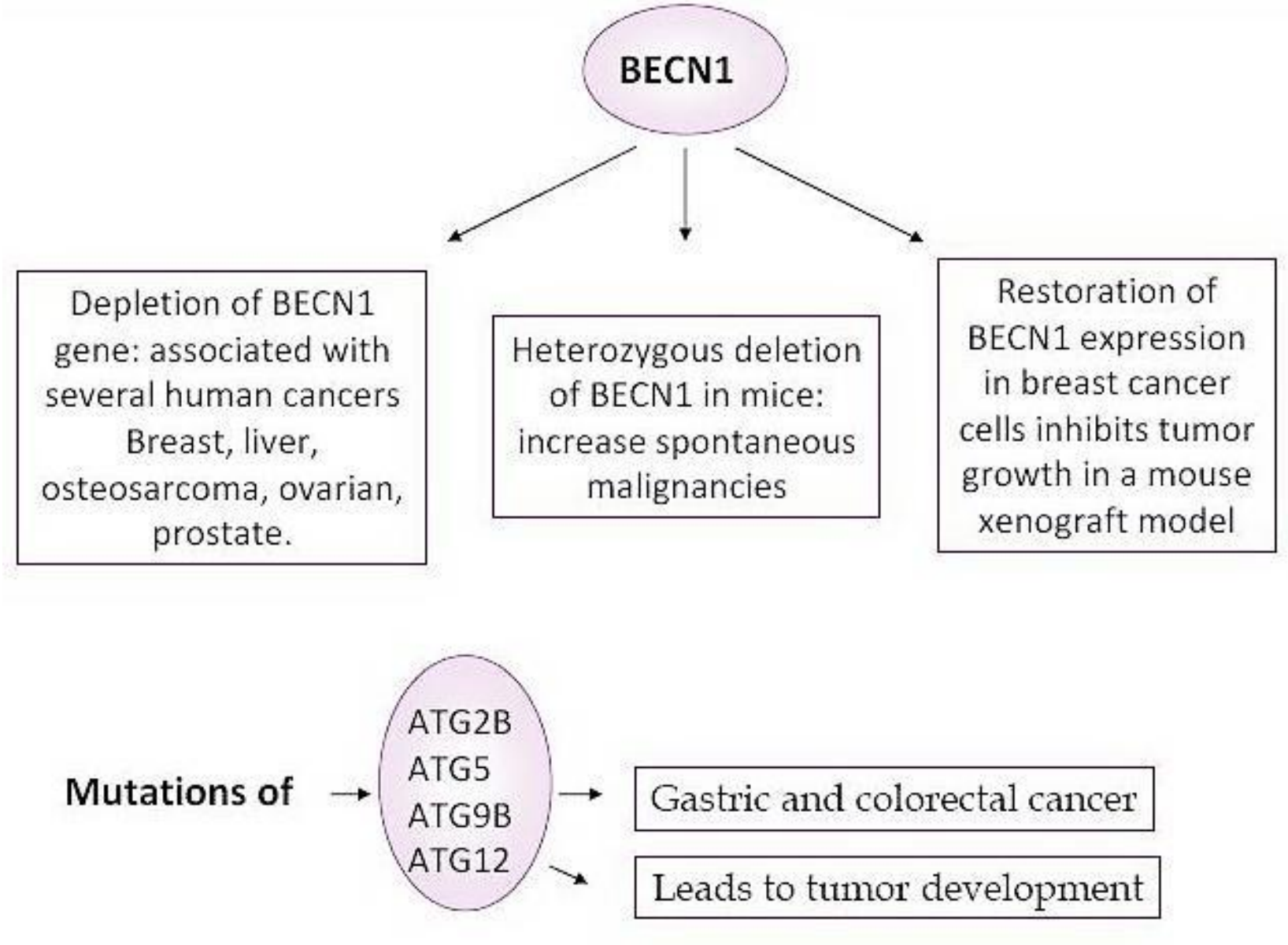


Maintain cellular Homeostasis

Prevent DNA damage and genomic instability

Protect tumor against necrosis and inflammation

Autophagy as Tumor Suppressor Mechanism



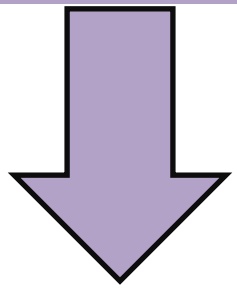
Autophagy as Tumor Suppressor Mechanism

Molecular Mechanisms

Autophagy

- protects cells against DNA damage and genomic instability by removing from the cytoplasm damaged organelles and proteins (major sources of ROS)
- may prevent tumor development by regulating the cellular level of p62
- may restrict the cell proliferation of transformed cells by activating oncogene induced senescence
- Could act as tumor suppressor as a non-autonomous mechanism by preventing necrosis and subsequent inflammation

Autophagy as Tumor Promoting Mechanism



Allow tumor cells to survive under stressful conditions

Sustain the deep metabolic reorganization that cancer cells encounter after oncogenic transformation

Support tumor development by maintaining the survival and self renewal of cancer stem cells

Autophagy as Tumor Promoting Mechanism

Autophagy is necessary for tumor progression

BECN1

Always monoallelically deleted

Tumor cells require functional autophagy for a malignant transformation to occur

ATG5
ATG7

Deletion: abolishes tumor growth in a RAS transformed model

The only tumors developped by mice harboring an hepatic deletion are only benign tumors

Autophagy as Tumor Promoting Mechanism

1- Autophagy allow cancer cell to survive despite metabolic stress

- In solid tumors, autophagy is localized in hypoxic regions of the tumors, its inhibition induce cell death.
- Due to increased cell proliferation, cancer cells have a high demand for nutrient and oxygen.

2- Autophagy participate in the tumor cell dormancy

- some cells enter into a senescent/dormant state (re-enter the cell cycle after a variable period of senescence).
- tumor recurrence.

3- Autophagy promotes the tumor cell dissemination and metastasis by protecting them from anoikis.

4- Metabolic reprogramming (warburg effect)

- inhibition of autophagy reduces glucose metabolism.
- mitochondrial metabolism: autophagy provide substrate for the TCA cycle (amino acids, lipids, sugars).

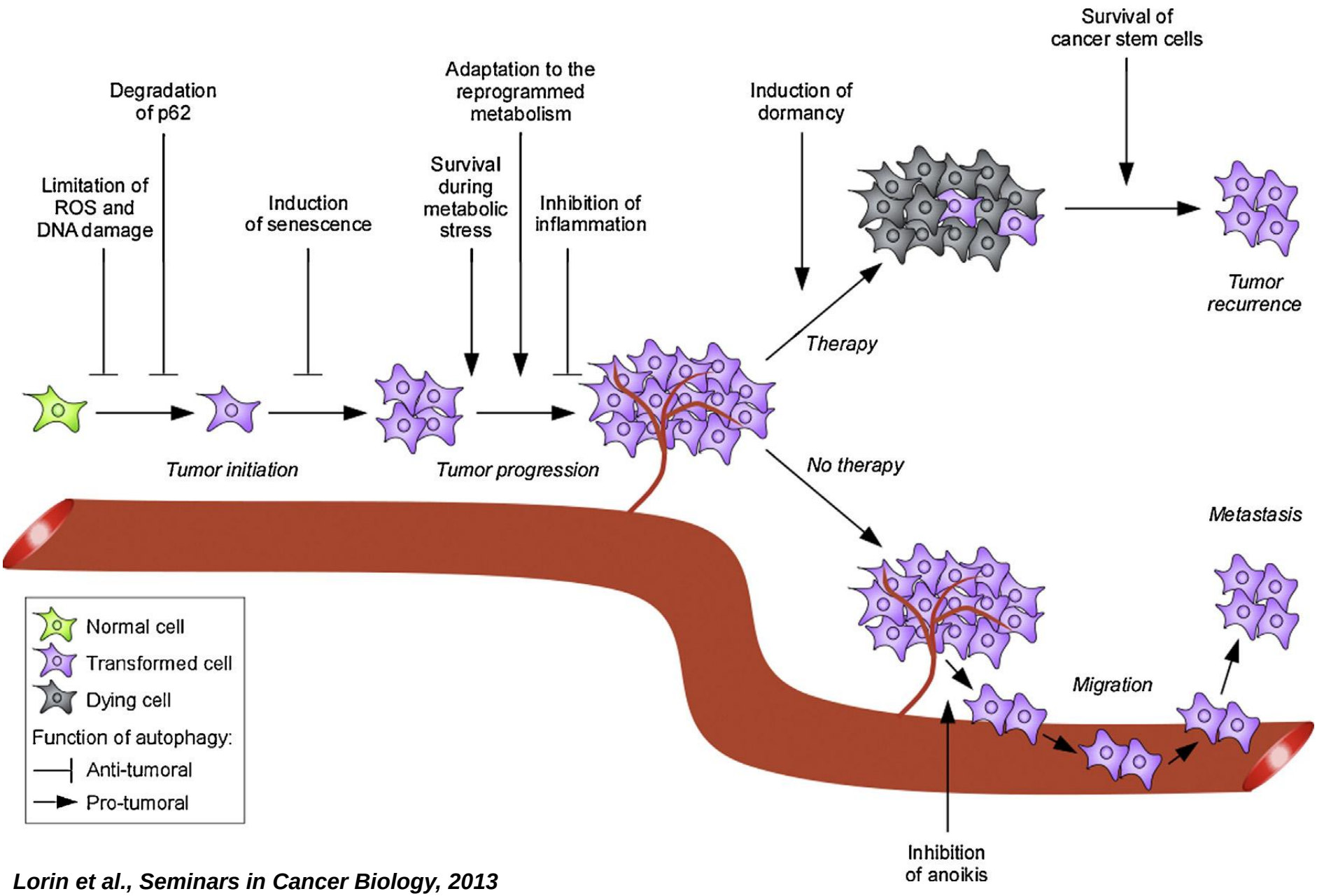
5- Cancer stem cells maintenance.

Autophagy and Cancer

Autophagy gene profile in human tumors

Human cancer types	Genetic autophagy modulation
Breast	BECN1 gene monoallelically deleted in up to 50% of cases. Loss of heterozygosis and aberrant methylation of promoter and the intron 2 in breast tumor tissues Association between BECN1 loss and HER2/NEU amplification
Colorectal	Frameshift mutations of ATG2B, ATG5 and ATG9B detected in a subset of MSI-H cases
Gastric	Frameshift mutation of UVRAG detected in 9.4% of MSI-H cases Frameshift mutations of ATG2B, ATG5 and ATG9B detected in a subset of MSI-H cases
Head and neck	ND
Liver	Reduction of Beclin 1 mRNA expression observed in 45.5% of HCC tissues
Leukemia	RAB7A gene rearrangement and deletion
Melanoma	Constitutive formation of autophagosomes detected in invasive and metastatic melanoma cells Melanoma cells actively undergoing autophagy Downregulation of Beclin 1 and LC3 during disease progression
Osteosarcoma	Weaker Beclin 1 IHC staining in tumors than in normal bones
Ovarian	BECN1 gene monoallelically deleted in up to 75% of case
Pancreatic	Positive LC3 IHC staining in 43.7% of cases
Prostate	BECN1 gene monoallelically deleted in up to 40% of cases

Suppressing and Promoting Roles of Autophagy during Tumorigenesis



Lorin et al., Seminars in Cancer Biology, 2013

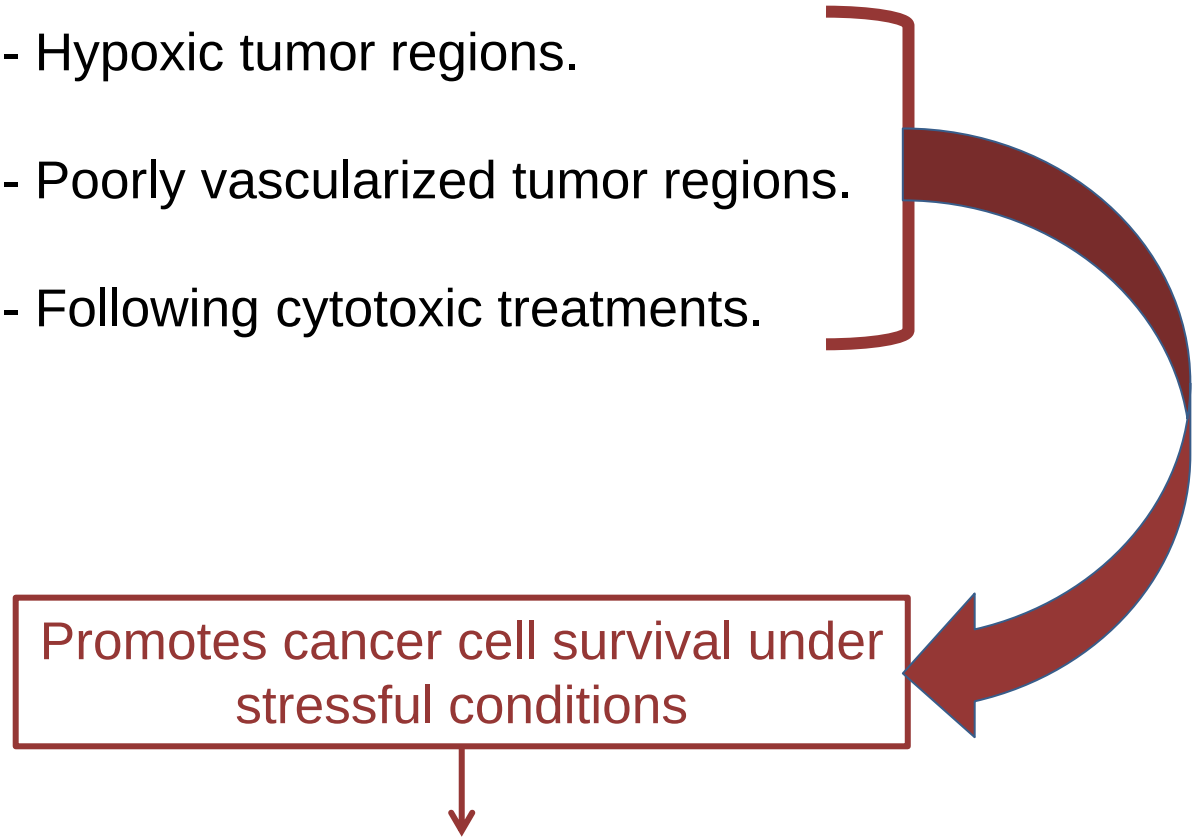
Autophagy in Cancer Treatment

Autophagy induction have been found to spatially localize to:

1- Hypoxic tumor regions.

2- Poorly vascularized tumor regions.

3- Following cytotoxic treatments.



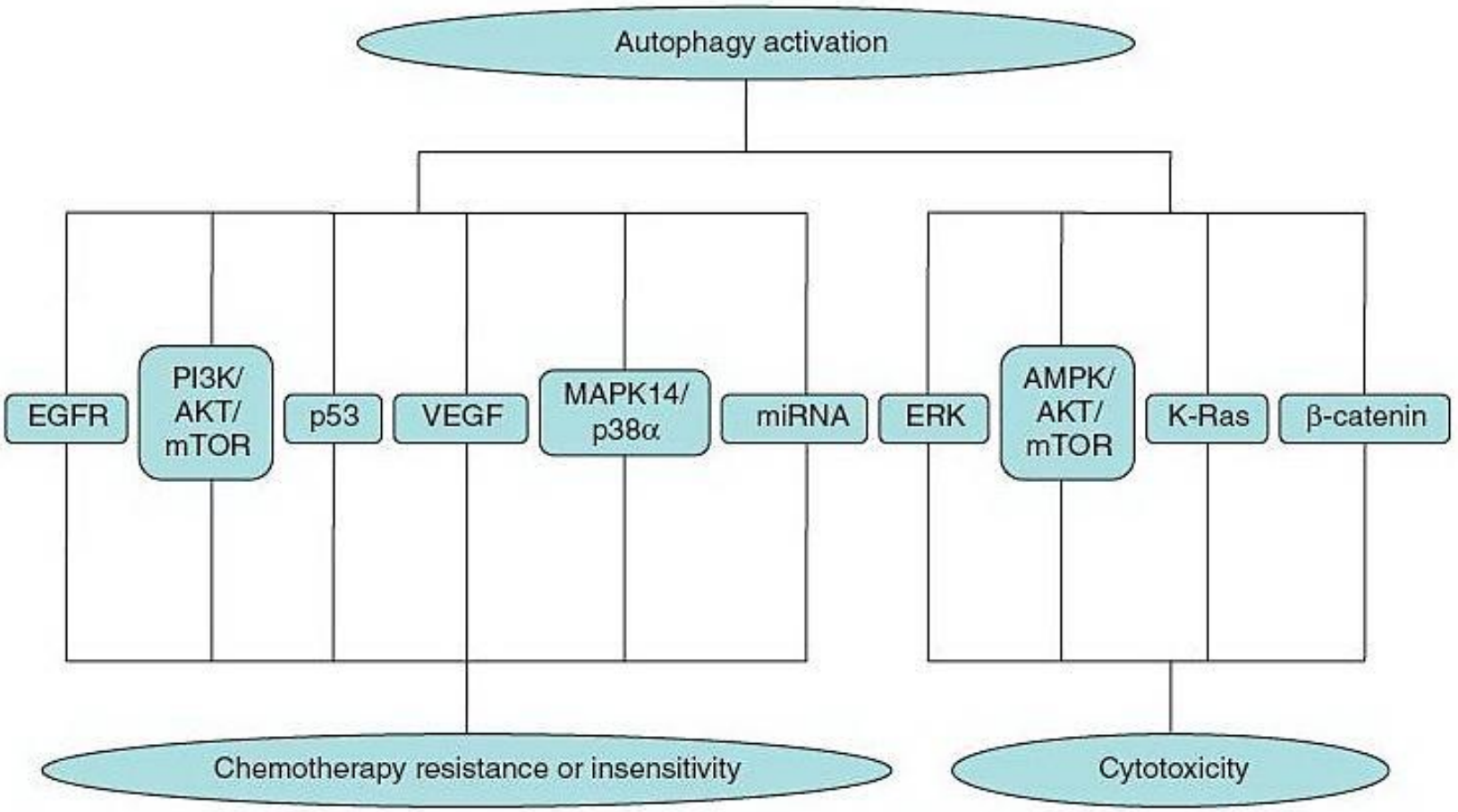
Autophagy in Cancer Treatment

Therapeutic Agent	Model	Autophagy Inhibition	Response
Temozolomide	Human malignant glioma cell lines	3-Methyladenine Bafilomycin A	Decreased cytotoxicity 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 Wortmannin	Increased cytotoxicity
Cisplatin	Human cervical cancer cell line (HeLa)	3-Methyladenine 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 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 3-Methyladenine	Increased apoptosis
Topotecan	Human NSLC cell line (A549)	Chloroquine	Increased cytotoxicity
Camptothecin	Human breast cancer cell lines	Wortmannin 3-Methlyadenine 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 Bafilomycin A	Decreased cytotoxicity Increased cytotoxicity
Imatinib	Human Philadelphia chromosome positive CML cells	Chloroquine	Increased cytotoxicity
HDACi/vorinostat	Human colon cancer cells and xenografts	Chloroquine	Increased cytotoxicity Decreased growth
HDACi/panobinostat	Human triple negative breast cancer cells and xenografts	Chloroquine	Increased cytotoxicity 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 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 Chloroquine 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 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 Bafilomycin A	Decreased cytotoxicity Increased cytotoxicity
Bortezomib	Human hepatocellular carcinoma cell lines and xenografts	Chloroquine	Increased apoptosis

Autophagy in Cancer Treatment



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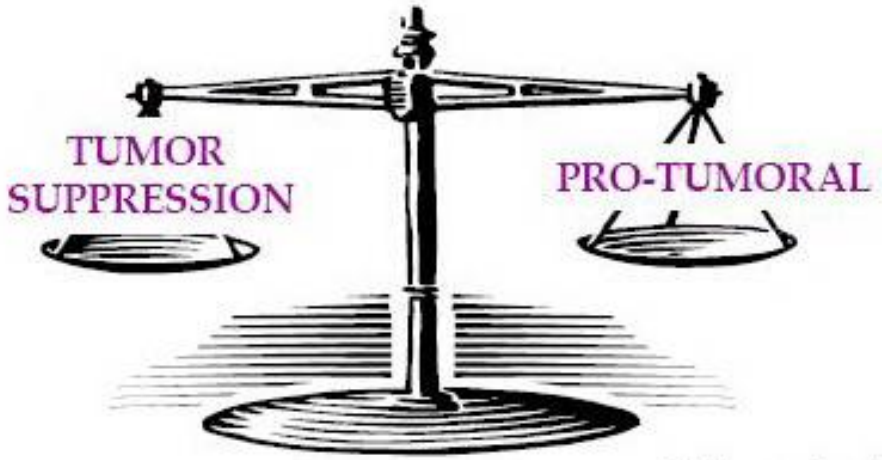
Rank	Status	Study
1	Unknown †	Autophagy Inhibition Using Hydrochloroquine in Breast Cancer Patients **Condition:** Breast Cancer **Intervention:** Drug: Hydrochloroquine
2	Recruiting	Modulation of Autophagy in Patients With Advanced/Recurrent Non-small Cell Lung Cancer - Phase II **Conditions:** Non-small Cell Lung Cancer; Advanced Non-small Cell Lung Cancer; Recurrent Non-small Cell Lung Cancer **Interventions:** Drug: Paclitaxel; Drug: Carboplatin; Drug: Hydroxychloroquine; Drug: Bevacizumab
3	Active, not recruiting	Hydroxychloroquine in Blocking Autophagy in Patients With Prostate Cancer Undergoing Surgery or Active Surveillance **Condition:** Prostate Carcinoma **Interventions:** Drug: Hydroxychloroquine; Other: Laboratory Biomarker Analysis
4	Completed	Chloroquine as an Anti-Autophagy Drug in Stage IV Small Cell Lung Cancer (SCLC) Patients **Condition:** Small Cell Lung Cancer **Intervention:** Drug: Chloroquine, A-CQ 100



Cell growth control
Quality control
Limitation of ROS
Limitation of genomic instability
Autophagic cell death
Antitumoral immunity
Senescence
Inhibition of chronic inflammation

Basal autophagy ?

Cell survival
⇨ oxygen and nutrients deprivation
⇨ chemotherapy
Chemoresistance
Prevention of apoptosis
Provides nutrient essential for rapid growth
Dormancy
Cancer stem cell survival

Induced autophagy ?

OPEN

Citation: Cell Death and Disease (2016) 7, e2205; doi:10.1038/cddis.2016.93

www.nature.com/cddis

Tumor suppression in mice lacking GABARAP, an Atg8/LC3 family member implicated in autophagy, is associated with alterations in cytokine secretion and cell death

FS Salah[1,2], M Ebbinghaus[3], VY Muley[4,5], Z Zhou[6], KRD Al-Saadi[2], M Pacyna-Gengelbach[7], GA O'Sullivan[8], H Betz[8,9], R König[4,5], Z-Q Wang[6,10], R Bräuer[1] and I Petersen*[,1]

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Cell Death and Disease (2016) 7, e2205; doi:10.1038/cddis.2016.93; published online 28 April 2016

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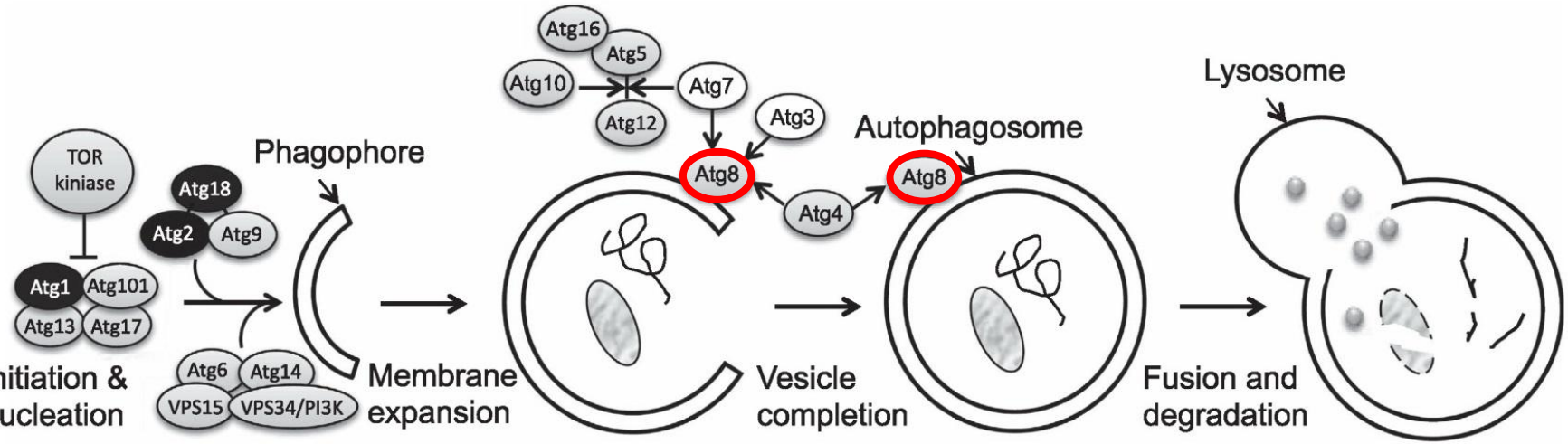
Gamma (γ)-aminobutyric acid type A ($GABA_A$) receptor-associated protein (**GABARAP**) is an evolutionary highly conserved gene family from yeast to mammals.

- 100% identity at amino acid level for mammalian forms.
- GABARAP regulates the intracellular trafficking of $GABA_A$ receptor, a major inhibitory neurotransmitter in cortical neurons.
- In mammals, there are several Atg8 homologues; grouped into two subfamilies:
  - LC3 (microtubule-associated protein-1 light chain 3)
  - GABARAP



Introduction: GABARAP

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- Neuroblastoma: ↓ GABARAP expression associated with ↓ survival
- Breast tumors: ↓ GABARAP expression
- Thyroid tumors: ↑ GABARAP expression in adenomas and thyroid cancer
- Colorectal tumors: ↑ GABARAP expression associated with a low grade of differentiation and shortened survival

However, the precise role of GABARAP in tumorigenesis is unknown so far

Introduction: Atg8/GABARAP and cell death

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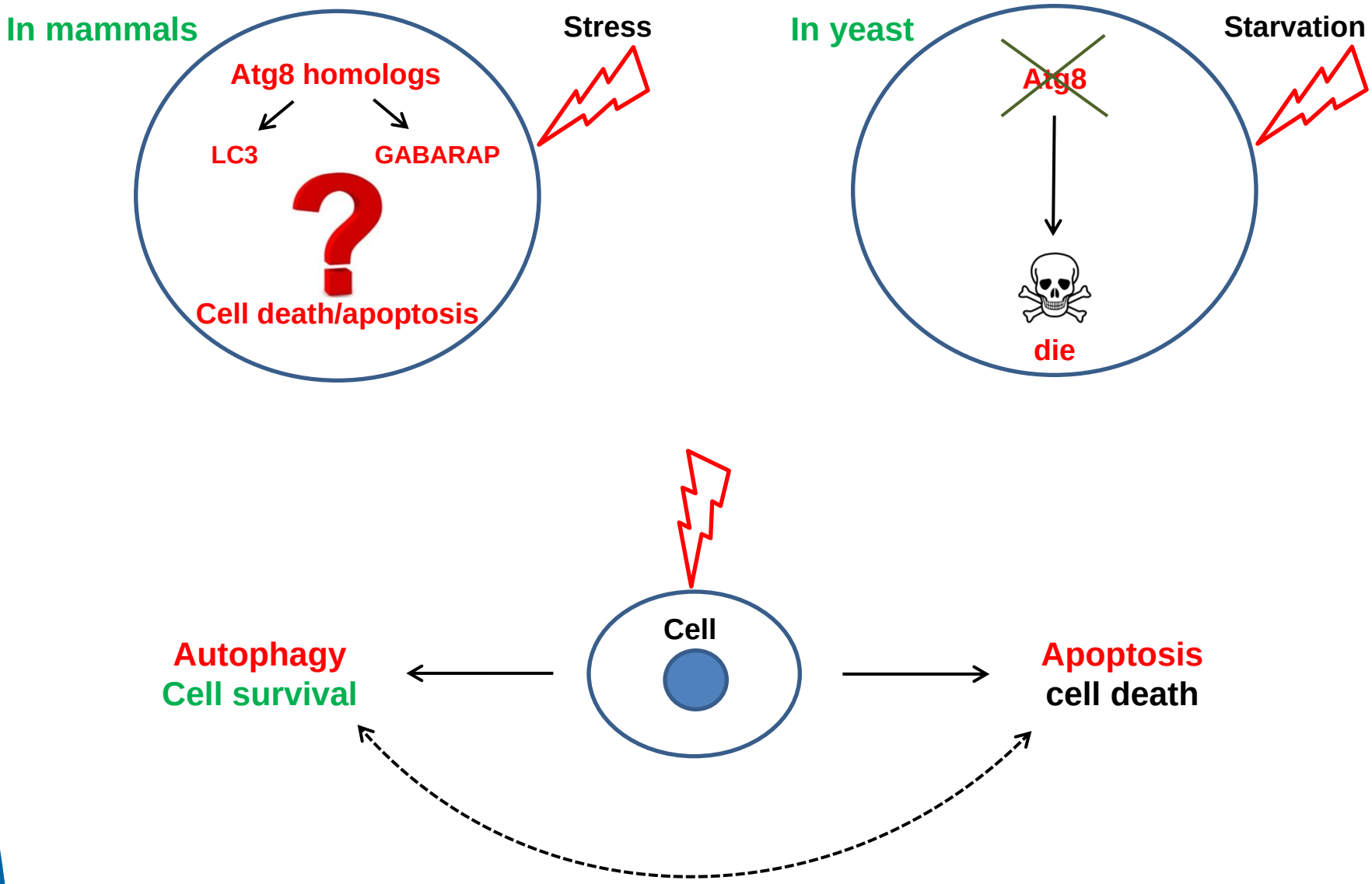

Explore the role of GABARAP in tumorigenesis by using a knockout mouse model

- **Treatment of GABARAP knockout mice with carcinogens affect tumorigenesis?**
- **GABARAP knockout mice influence the growth of inoculated tumor cells?**
- **What are the cellular mechanisms being affected by GABARAP deficiency?**
  - **Apoptosis**
  - **Immunity**

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Wild-type (C57BL/6) mice

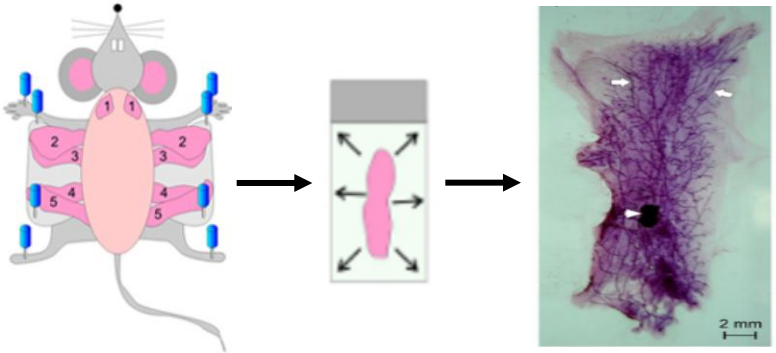
GABARAP knockout mice (C57BL/6 background)

Primary culture (MEFs)
Protein expression (WB)

Tumor inoculation
B16 melanoma cell

Carcinogen treatment (DMBA)

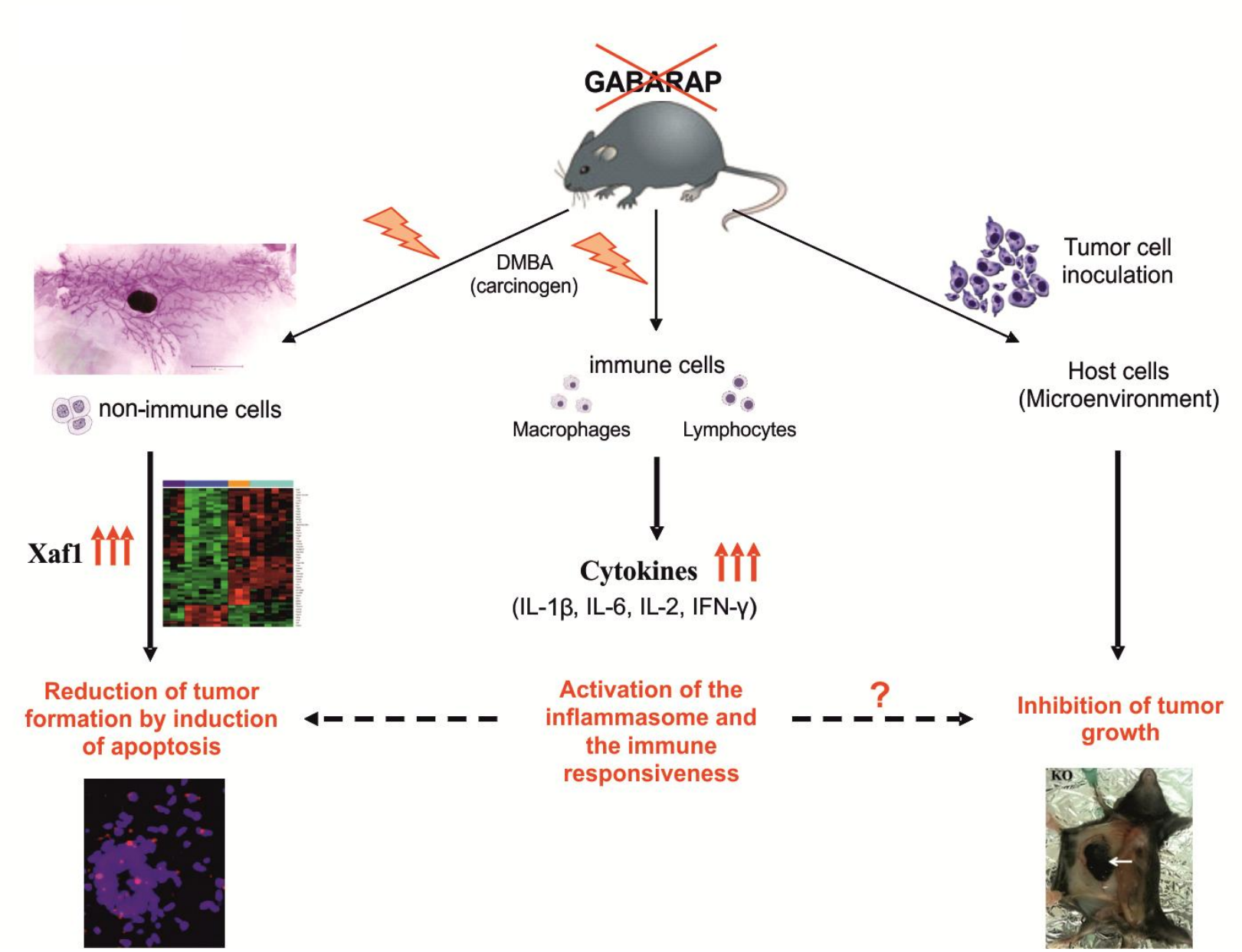
- Whole mount analysis of mammary glands



- Proliferation assay (IHC) by using Ki-67
- Apoptosis assay (TUNEL)
- cDNA microarray gene expression profiling
- qRT-PCR

Immunologic experiments:

- Cell surface marker analysis (FACS)
- Macrophages isolation
- Lymphocytes isolation
- Cytokine analysis (ELISA)


GABARAP
DMBA
(carcinogen)
Tumor cell
inoculation
non-immune cells
immune cells
Macrophages
Lymphocytes
Host cells
(Microenvironment)
Xaf1
Cytokines
(IL-1β, IL-6, IL-2, IFN-γ)
Reduction of tumor formation by induction of apoptosis
Activation of the inflammasome and the immune responsiveness
?
Inhibition of tumor growth
KO

Thank you for attention