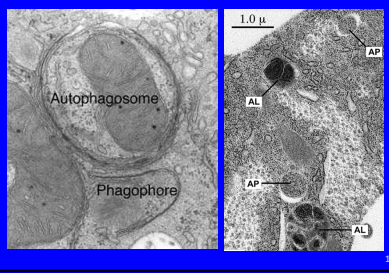
WHAT IS AUTOPHAGY ?

- LYSOSOMAL PATHWAY OF MACROMOLECULAR DEGRADATION TRIGGERED BY CELL DAMAGE (membrane oxidation, unfolded protein aggregates, aged structures)
- UPREGULATED DURING STARVATION ('sensors' for aminoacids concentration)

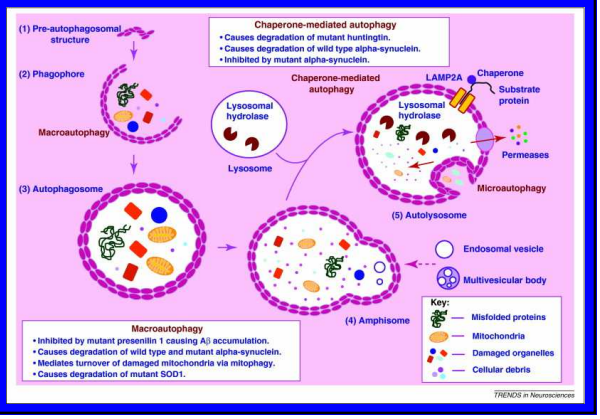
...first described by De Duve in 1963

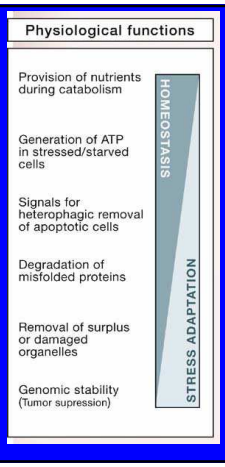
(Nobel Prize)



1

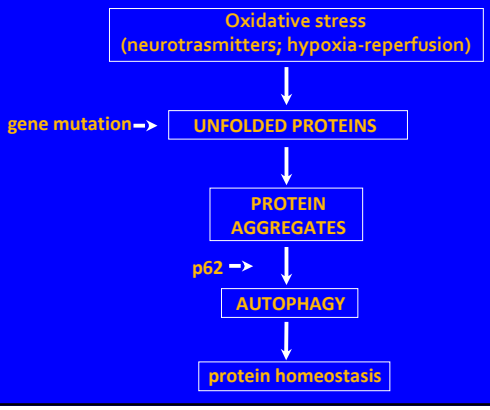
Three types of AUTOPHAGY





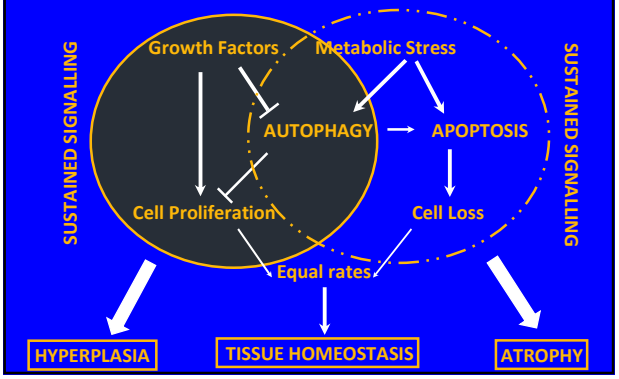
3

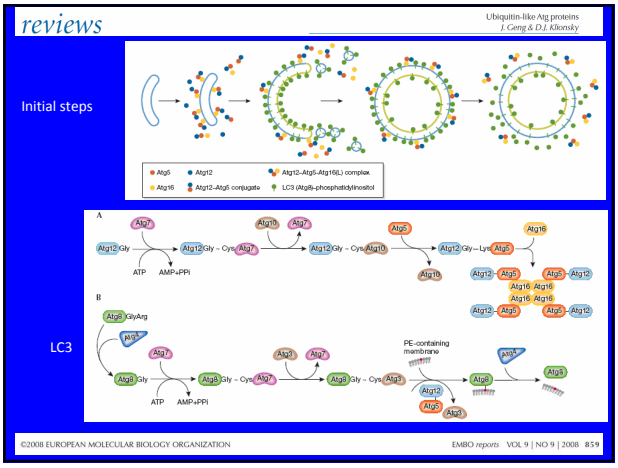
PROTEIN HOMEOSTASIS



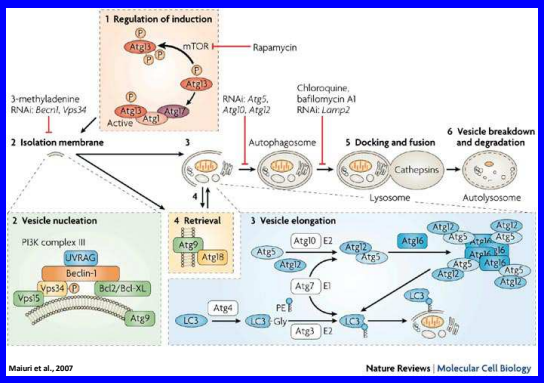
4

TISSUE HOMEOSTASIS





THE CANONICAL PATHWAY

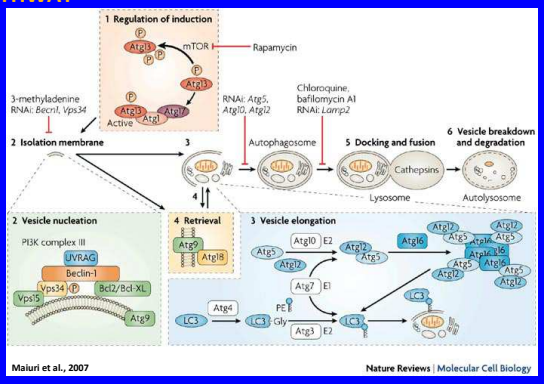


Recognition of Substrate

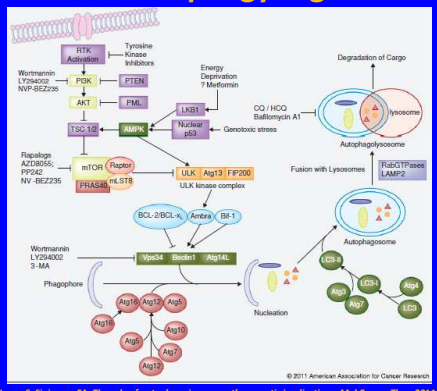
ROLE OF
p62/SQSTM

p62 and NBR1 can mediate the selective entrapment of autophagic substrates, functioning as cargo receptors for ubiquitinated protein aggregates and organelles. These proteins contain in fact the LC3-interacting sequence WXXL/I which can serve for the targeting to the autophagosome of the bound substrate.

MANIPULATION OF THE AUTOPHAGY-LYSOSOMAL PATHWAY



How is autophagy regulated



Yang ZJ, Chee CE, Huang S, Sinicrope FA. The role of autophagy in cancer: therapeutic implications. Mol Cancer Ther. 2011 Sep;10(9):1533-41.

REGULATORY PATHWAYS

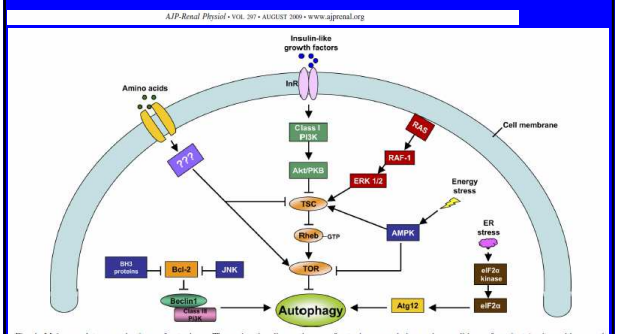
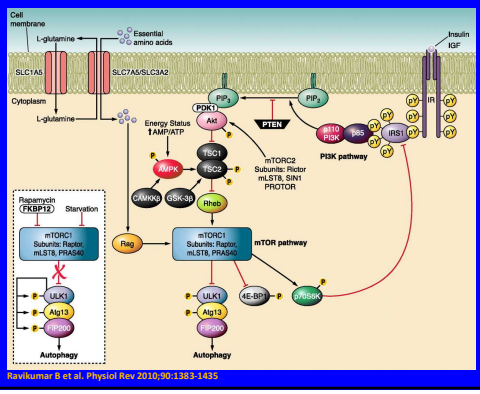


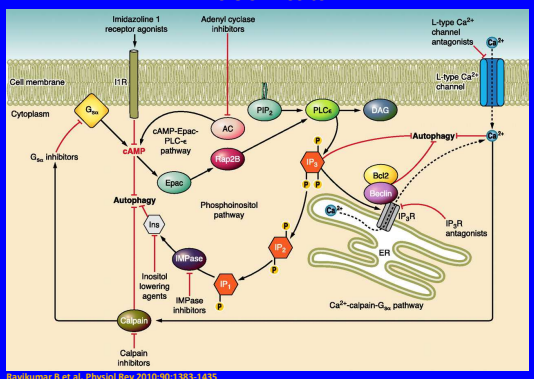
Fig. 4. Major regulatory mechanisms of autophagy. The major signaling pathways of autophagy regulation under conditions of nutrient (amino acids, growth factors) deprivation, endoplasmic reticulum (ER) stress, energetic stress or depletion (see detailed discussion in text) are depicted. The pathways may cross talk and regulate autophagy at different levels, including induction and expansion of the isolation membrane, enclosure of the isolation membrane to form autophagosome, and fusion with lysosome. PI3K, PI3-kinase; AMPK, AMP-dependent protein kinase; TSC, tuberous sclerosis complex; eIF-2α, eukaryotic initiation factor-2α; InR, insulin receptor; RAS, rat sarcoma (a protein encoded by rat sarcoma virus oncogene).

The phosphatidylinositol 3-kinase (PI3K)-mammalian target of rapamycin (mTOR) pathway regulating autophagy



Ravikumar B et al. Physiol Rev 2010;90:1383-1435

mTOR-independent pathway regulating mammalian autophagy: role of Inositol



Ravikumar B et al. Physiol Rev 2010;90:1383-1435

Oncogenes-Oncosuppressors

ONCOGENE

ONCOSUPPRESSOR

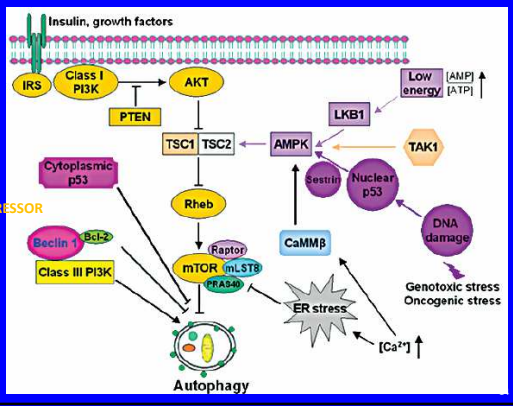


TABLE 1. ONCOSUPPRESSOR PROTEINS WITH AUTOPHAGY-STIMULATORY PROPERTIES

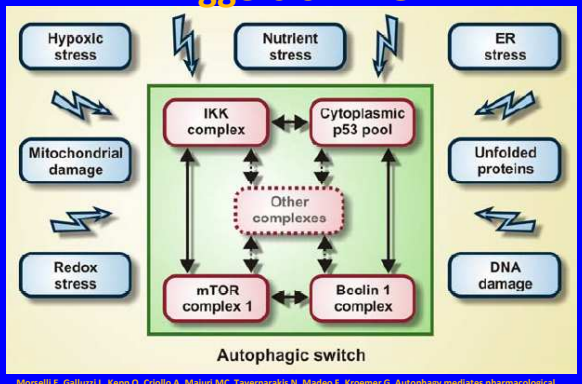
Protein(s)	*Link(s) to oncogenesis*	*Link(s) to autophagy*	*References*
ATG4C	ATG4C-deficient mice exhibit an increased sensitivity to chemically induced tumors	Cysteine protease that participates in the conversion of LC3-I into LC3-II	(160, 228)
Beclin 1	Deleted in a consistent fraction of mammary, ovarian, and prostate cancers; downregulated at the protein level in brain, gastric, and colorectal cancer	Essential component of the hVPS34-containing class III PI3K complex	(3, 140, 171, 217, 258)
BH3-only proteins	Mutated or underexpressed in multiple human tumors (e.g., melanoma, renal carcinoma)	Stimulate autophagy by releasing Beclin 1 from BCL-2/BCL-X_L–mediated inhibition	(131, 146, 153, 246, 255, 262)
DIRAS (ARH0)	RAS-related tumor suppressor downregulated in >60% ovarian cancers	Negatively regulates mTOR via PI3K signaling and upregulates ATG4	(5, 56, 147)
SH3GLB1 (BIF1)	Downregulated in colorectal adenocarcinoma, knockout of SH3GLB1 enhances the development of spontaneous tumors in mice	Positive modulator of the hVPS34-containing class III PI3K complex	(37, 224)
DAPK1	Often silenced in human tumors by epigenetic mechanisms (promoter hypermethylation)	Interacts with MAP1B, may induce the activation of p53, favors the release of Beclin 1 from BCL-2/X_L	(29–32, 87, 162, 259, 260)
NF1	Loss-of-function mutations lead to type I neurofibromatosis	Negative regulator of RAS signaling with indirect proautophagic effects	(33, 34, 103, 215)
$p19^{ARF}$	Mutated or lost in multiple types of human cancer (e.g., lymphoma, NSLC, breast cancer)	Inhibits MDM2 in the nucleolus, thereby allowing for stabilization of nuclear p53	(2, 200, 201, 207)
p150 (?)	Overexpressed in cervical carcinoma	Essential component of the hVPS34-containing class III PI3K complex	(157, 247, 254)
p53	Mutated in >50% human cancers	Controversially regulates autophagy, depending on its subcellular localization	(67, 68, 154, 178, 221)
PTEN	Germline mutations cause Cowden's and Bannayan-Riley-Ruvalcaba's diseases; somatically mutated/silenced in many cancers	Phosphatase that antagonizes the activity of PI3K, thereby inhibiting mTOR	(7, 45)
RAB7A	Rearranged in several types of leukemia	Small GTP-binding protein that plays a role in autophagosome maturation	(51, 54, 99, 109)
smARF	Mutated or lost in multiple types of human cancer (e.g., lymphoma, NSLC, breast cancer)	Acts at mitochondria by releasing Beclin 1 from BCL-2/BCL-X_L–mediated inhibition	(2, 200, 201, 207)
STK11 (LKB1)	Germline mutations cause Peutz-Jeghars's syndrome; somatic ones are found in NSCLC	AMPK activator; may also promote autophagy by stabilizing $p27^{KIP1}$	(38, 88, 100, 141, 216, 248)
TSC1 TSC2	Germline mutations cause TSC	TSC1/TSC2 stimulates the GTPase activity of RHEB, thus inhibiting mTOR	(73, 96, 97, 236, 263)
UVRAG	Often monoallelically deleted in colon cancer; affected by frameshift mutations in gastric carcinomas with microsatellite instability	Positive modulator of the hVPS34-containing class III PI3K complex	(113, 137, 138)

TABLE 2. ONCOPROTEINS WITH AUTOPHAGY-INHIBITORY FUNCTIONS

Protein(s)	*Link(s) to oncogenesis*	*Link(s) to autophagy*	*Reference*
AKT1 (PKB)	Gain-of-function mutations or amplifications characterize a high fraction of human cancers	Constitutively active AKT1 functions as an autophagy inhibitor *in vitro* and *in vivo*	(14, 158, 254)
BCL-2 BCL-X_L	Overexpressed in a plethora of human cancers, notably in hematological malignancies	Inhibit autophagy by sequestering Beclin 1 into inactive complexes	(107, 155, 195)
PDPK1	Gain-of-function mutations or amplifications are found in a high fraction of human tumors	PIP_3-responsive kinase that inhibits the TSC1/TSC2	(197, 213)
PI3K (class I)	Gain-of-function mutations or amplifications are common to many human cancers	Generates PIP_3, which indirectly activates the antiautophagic functions of mTOR	(192, 194, 211, 257)
RAS (?)	Hyperactivated in a relevant proportion of human cancers all confounded	Signal transducer of the RTK-mTOR pathway with dual roles in autophagy regulation	(64, 170, 193)

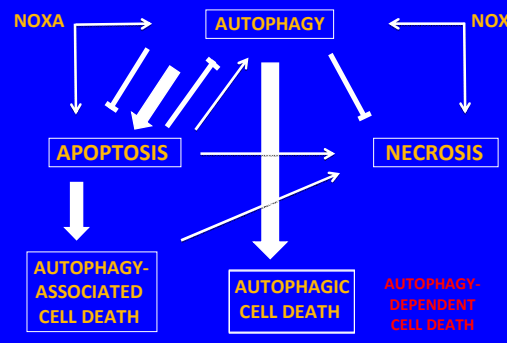
Morselli E, Galluzzi L, Kepp O, Mariño G, Michaud M, Vitale I, Maiuri MC, Kroemer G. Oncosuppressive functions of autophagy. Antioxid Redox Signal. 2011 Jun;14(11):2251-69.

Triggers of ATG

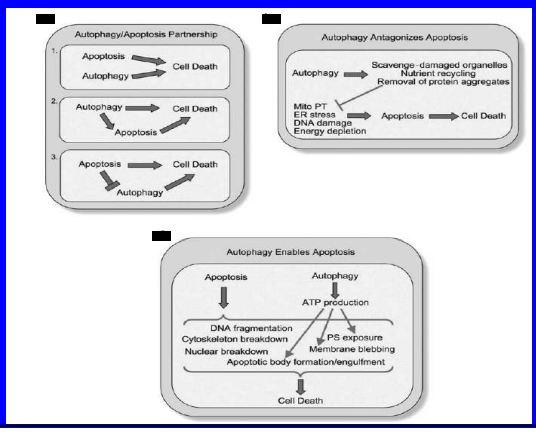


Morselli E, Galluzzi L, Kepp O, Criollo A, Maiuri MC, Tavernarakis N, Madeo F, Kroemer G. Autophagy mediates pharmacological lifespan extension by spermidine and resveratrol. Aging (Albany NY). 2009 Dec 23;1(12):961-70.

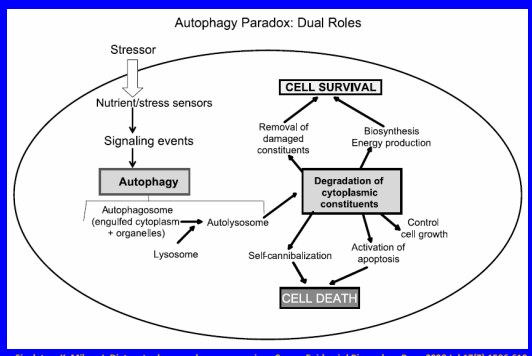
COMPLEX INTERPLAY BETWEEN CELL DEATH PATHWAYS



THE COMPLEX CROSS-TALK BETWEEN AUTOPHAGY AND APOPTOSIS

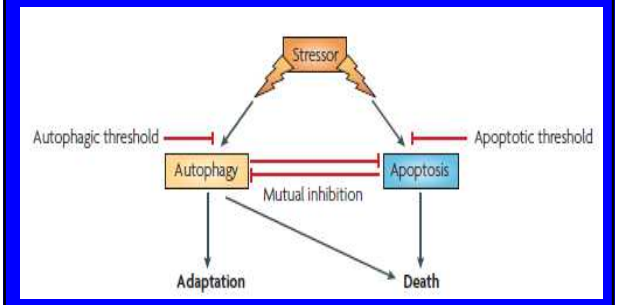


DUAL ROLES OF AUTOPHAGY IN CELL SURVIVAL AND DEATH



Singletary K, Milner J. Diet, autophagy, and cancer: a review. Cancer Epidemiol Biomarkers Prev. 2008 Jul;17(7):1596-610.

The relationship between apoptosis and autophagy



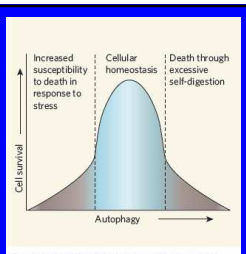


Figure 4 | Relationship between the levels of autophagy and cell death. Physiological levels of autophagy are essential for normal cellular homeostasis. The absence of autophagy increases cell death during metabolic stress and on treatment with cytotoxic chemotherapeutic agents. By contrast, excessive levels of autophagy promote cell death, presumably via self-cannibalization. Antitumour effects may be observed at all levels of autophagy, in relation either to cell death (when autophagy is very low or very high) or to cell-death-independent tumour-suppressor effects (when autophagy levels are intermediate).

Levine B. Cell biology: autophagy and cancer. Nature. 2007 Apr 12;446(7137):745-7.

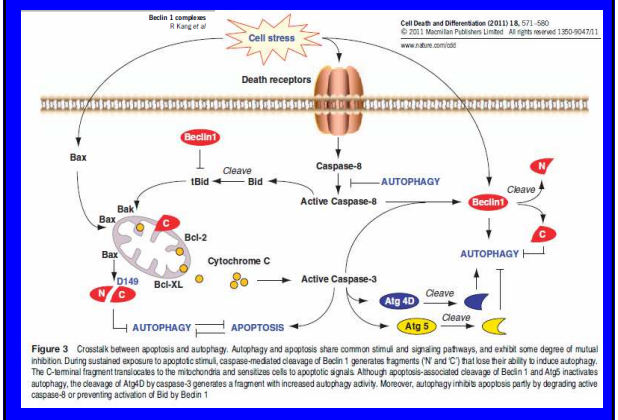


Figure 3 Crosstalk between apoptosis and autophagy. Autophagy and apoptosis share common stimuli and signaling pathways, and exhibit some degree of mutual inhibition. During sustained exposure to apoptotic stimuli, caspase-mediated cleavage of Beclin 1 generates fragments ('N' and 'C') that lose their ability to induce autophagy. The C-terminal fragment translocates to the mitochondria and sensitizes cells to apoptotic signals. Although apoptosis-associated cleavage of Beclin 1 and Atg5 inactivates autophagy, the cleavage of Atg4D by caspase-3 generates a fragment with increased autophagy activity. Moreover, autophagy inhibits apoptosis partly by degrading active caspase-8 or preventing activation of Bid by Beclin 1

AUTOPHAGY- APOPTOSIS CROSS - TALK

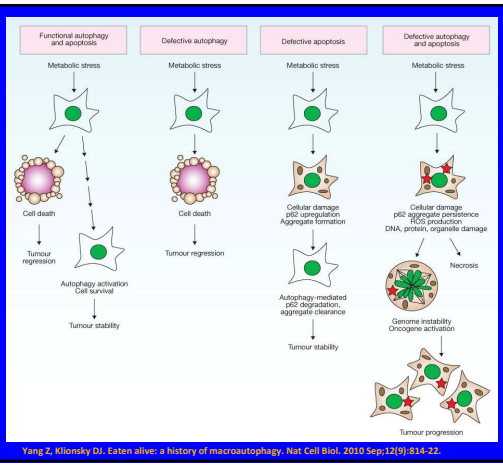
- ✓ AUTOPHAGY prevents APO ✓(degradation of leaking mtc)
- ✓ APOPTOSIS inhibits ATOPHAGY Bcl2 ⟙ beclin-1; Bcl2 ⟙ Ca++ ↑; p53 ⟙
- ✓ AUTOPHAGY enables APO Atg5t ⟙ bcl2
- ✓ APO induces ATOPHAGY p53 ⟙ DRAM

… and more:

caspase 3 cleaves Atg4 (which processes LC3);

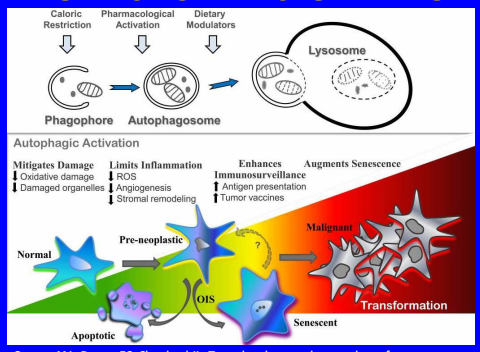
caspases 3 and 7 cleave beclin 1, whose C-term promotes mtc permeablization;

FLIP, which inhibits casp 8, suppresses autophagy by competing with LC3 for binding to Atg3



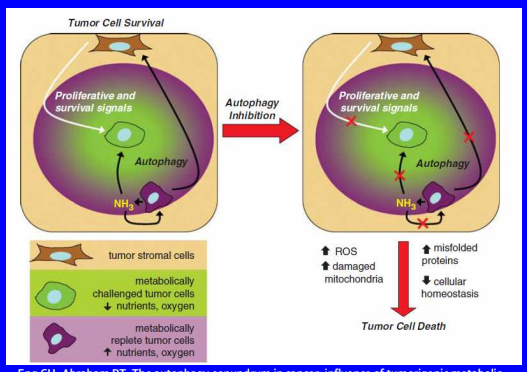
Yang Z, Klionsky DJ. Eaten alive: a history of macroautophagy. Nat Cell Biol. 2010 Sep;12(9):814-22.

THE ROLE OF AUTOPHAGY IN PREVENTING NEOPLASTIC TRANSFORMATION



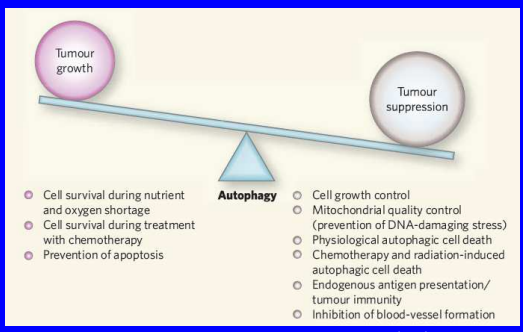
Steeves MA, Dorsey FC, Cleveland JL. Targeting the autophagy pathway for cancer chemoprevention. Curr Opin Cell Biol. 2010 Apr;22(2):218-25.

ROLE OF STROMA CELL-TUMOUR CELL INTERACTION



Eng CH, Abraham RT. The autophagy conundrum in cancer: influence of tumorigenic metabolic reprogramming. Oncogene. 2011 Jun 13. doi: 10.1038/onc.2011.220.

CONFLICTING EFFECTS OF AUTOPHAGY ON CANCER



Levine B. Cell biology: autophagy and cancer. Nature. 2007 Apr 12;446(7137):745-7.

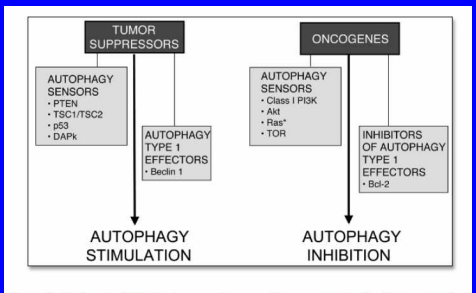
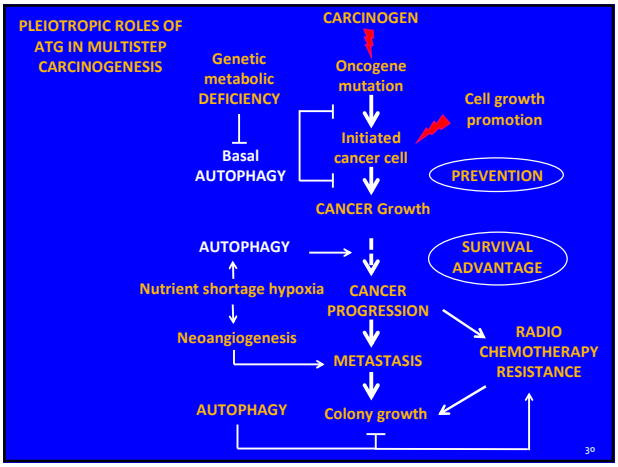
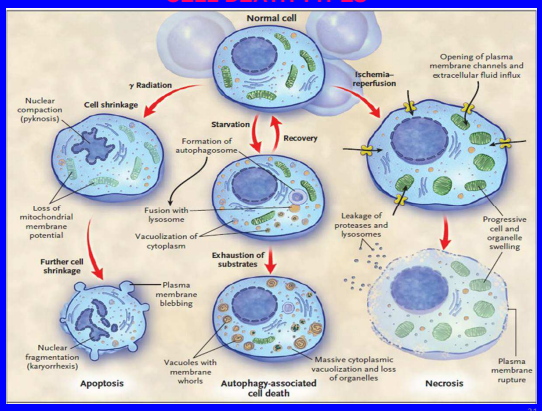


Figure 1. Dual control of autophagy and cancer. Shown are molecules that are involved in autophagy induction and tumor suppression (left side) and autophagy inhibition and oncogenesis (right side). These relationships form much of the basis for the hypothesis that autophagy is a tumor suppressor mechanism. *The oncoprotein Ras has been shown to both inhibit autophagy via activation of the class I PI3K pathway and stimulate autophagy via activation of the ERK1/2 pathway (see ref. 1).

Levine B. Unraveling the role of autophagy in cancer. Autophagy. 2006 Apr-Jun;2(2):65-6.



CELL DEATH TYPES



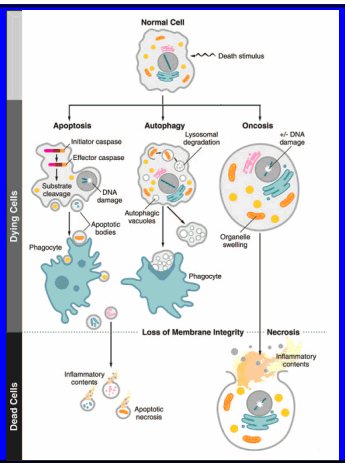
Hotchkiss et al., 2009

Cell Death and Differentiation (2007) 14, 1237–1266

www.nature.com/cdd

Table 2 Morphological aspects of different modes of cell death[1]

Cell death mode	Characteristic morphological aspects	Notes
Apoptosis (Type 1)	● Rounding up of the cell ● Reduction of cellular and nuclear volume (pyknosis) ● Retraction of pseudopodes ● Nuclear fragmentation (karyorrhexis) ● Little modification of cytoplasmic organelles ● Plasma membrane blebbing	'Apoptosis' is the original term introduced by Kerr *et al.*[44] to define a cell death with specific morphological features.
Autophagy (Type 2)	● Lack of chromatin condensation ● Massive vacuolization of the cytoplasm (double-membraned autophagic vacuoles)	'Autophagic cell death' defines cell death occurring *with* autophagy, though it may misleadingly suggest a form of death occurring *through* autophagy.[45]
Necrosis (oncosis) (Type 3)	● Cytoplasmic swelling ● Rupture of plasma membrane ● Swelling of cytoplasmic organelles ● Moderate chromatin condensation	'Necrosis' identifies, in a negative fashion, cell death lacking the features of apoptosis or autophagy, and usually appears as oncosis.[46]
Mitotic catastrophe	● Micronucleation ● Multinucleation	'Mitotic catastrophe' refers to a cell death occurring during or shortly after a failed mitosis.[11]



NECROSIS

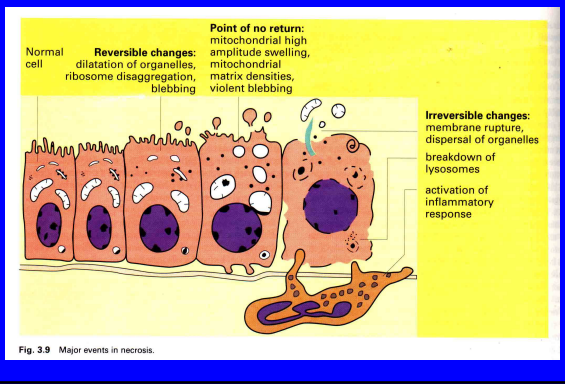


Fig. 3.9 Major events in necrosis.

APOPTOSIS

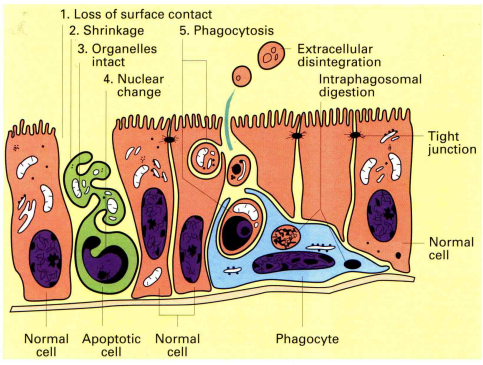
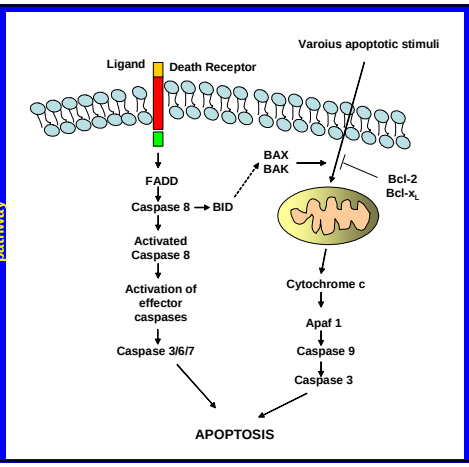
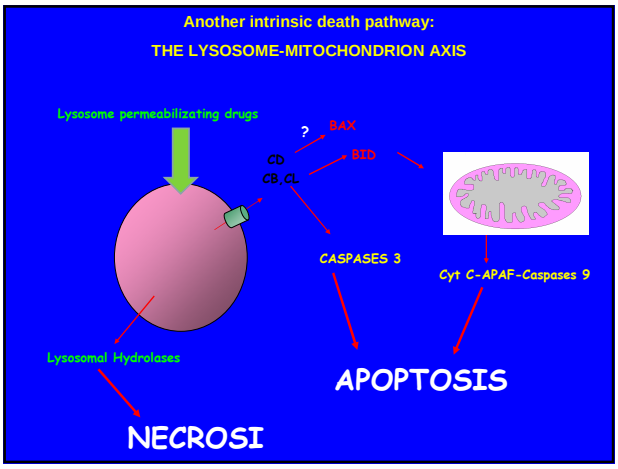


Fig. 3.1 Key morphological changes in apoptosis.




Another intrinsic death pathway:
THE LYSOSOME-MITOCHONDRION AXIS
Lysosome permeabilizating drugs
?
BAX
CD
CB, CL
BID
CASPASES 3
Cyt C-APAF-Caspases 9
Lysosomal Hydrolases
APOPTOSIS
NECROSI