

## Autophagy by Natural Products in Cancer Cells

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The physiological function of autophagy in normal cells is to maintain cellular homeostasis in nutrient-deprived states. The degradation of cytoplasmic material serves cell-autonomous energy production to promote cell survival. Hence, autophagy acts as pro-survival mechanism. Other autophagy-inducing metabolic stressors are growth-factor deprivation, hypoxia, and reactive oxygen species. Depending on the cellular context, autophagy also induces cell death. Therefore, autophagy has been described as type II-cell death, in contrast to apoptosis (type I-cell death). Cell death raised considerable interest in cancer pharmacology. Although natural products have been demonstrated to induce autophagy, the full therapeutic potential still needs to be explored.

Apoptosis has been one of the hottest topics in cell biology, immunology and pharmacology during the past two decades [1-3]. More recently, new forms of cell death were discovered such as necroptosis, immunogenic cell death, etc. Autophagy has also been described as a form of cell death, which can also act as pro-survival mechanism.

The term autophagy describes the degradation of intracellular cargo (mitochondria, proteins, other structures) within lysosomes. Three types of autophagy are known:

- 1) Macroautophagy is best analyzed as of yet, as is commonly termed "autophagy". Cargo delivery to lysosomes is mediated by vesicles with a membrane bi-layer. These vesicles ("autophagosomes") fuse with lysosomes ("autolysosome") to release intravesicular material.
- 2) Micro-Autophagy: Cytoplasmic material is taken up by lysosomes, by invagination of the lysosomal membrane.
- 3) Chaperone-mediated autophagy: Proteins form complexes with Hsc-70 or other chaperones, which are recognized by lysosomal associated membrane proteins (LAMP), and translocated into lysosomes.

Macroautophagy (hereafter, referred to as "autophagy" in this article) may be subclassified into "basal autophagy" (constitutive turnover of cytoplasmic components) and "induced autophagy" (degradation of intracellular cargo upon appropriate stimulation) [4].

Autophagy is described as type II -cell death in contrast to apoptosis (type I-cell death) [5], because it induces cell death depending on the cellular context in contrast to apoptosis (type I-cell death) [5]. Autophagy can precede, accompany or prevent apoptosis, and the molecular mechanisms of cross-talk between autophagy and apoptosis are still incompletely understood [6-11]. Autophagy and apoptosis may cooperate leading to cell death. In other cellular settings, autophagy may counteract apoptosis by creating a pro-survival cellular milieu. Finally, autophagy may enable apoptosis by providing energy to induce ATP-dependent apoptotic processes.

All forms of cell death have raised considerable interest in cancer pharmacology, since most if not all anticancer drugs induce apoptosis, or another form of cell death. Apoptosis represents an evolutionary

very old mechanism of organisms to cope with toxic insults, either from environment (e.g. heavy metals) or from poisonous ingredients in foods or microbial infections. In clinical oncology, it has however, frequently been overseen that natural products from plants, fungi or microorganisms induce cell death and thus, provide a basis for the development of novel treatment strategies in cancer therapy. It has been well documented in the literature that natural products induce apoptosis in cancer cells [12,13]. Although, a number of papers also demonstrated that natural products induce autophagy (Table 1), the full therapeutic potential still needs to be explored [14]. Given the fundamental importance of autophagy in cell biology, and the fact that natural products represent a major resource for the development of cancer drugs [15], cytotoxic compounds from nature certainly deserve more attention in cancer therapy dealing with autophagy regulation.

### References

1. Andersen MH, Becker JC, Straten PT (2005) Regulators of apoptosis: suitable targets for immune therapy of cancer. *Nat Rev Drug Discov* 4: 399-409.
2. Ocker M, Höpfner M (2012) Apoptosis-modulating drugs for improved cancer therapy. *Eur Surg Res* 48: 111-120.
3. Vyas VK, Chintia C, Pandya MR (2012) Biology and Medicinal Chemistry Approaches towards various apoptosis inducers. *Anticancer Agents Med Chem*.
4. Mizushima N (2007) Autophagy: process and function. *Genes & Dev* 21: 2861-2873.
5. Schweichel JU, Merker HJ (1973) The morphology of various types of cell death in prenatal tissues. *Teratology* 7: 253-266.
6. Efferth T, Giaisi M, Merling A, Krammer PH, Li-Weber M (2007) The anti-malaria herbal compound artesunate triggers mitochondria-mediated apoptosis in leukemia T cells by inducing oxidative stress. *PLoS ONE* 2: e693.
7. Eisenberg-Lerner A, Bialik S, Simon H-U, Kimchi A (2009) Life and death partners: apoptosis, autophagy and the cross-talk between them. *Cell Death Differ* 16: 966-975.
8. Luo M, Liu X, Zu Y, Fu Y, Zhang S, et al. (2010) Cajanol, a novel anticancer agent from *Pigeonpea* [*Cajanus cajan* (L.) Millsp.] roots, induces apoptosis in human breast cancer cells through a ROS-mediated mitochondrial pathway. *Chem Biol Interact* 188: 151-160.
9. Hamacher-Brady A, Stein HA, Turschner S, Toegel I, Mora R, et al. (2011) Artesunate activates mitochondrial apoptosis in breast cancer cells via iron-catalyzed lysosomal reactive oxygen species production. *J Biol Chem* 286: 6587-6601.

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10. Reichert S, Reinboldt V, Hehlhans S, Efferth T, Rödel C, et al. (2012) A radiosensitizing effect of artesunate in glioblastoma cells is associated with a diminished expression of the inhibitor of apoptosis protein survivin. *Radiother Oncol* 103: 394-401.
11. Zhang Y, Luo M, Zu Y, Fu Y, Gu C, et al. (2012) Dryofragin, a phloroglucinol derivative, induces apoptosis in human breast cancer MCF-7 cells through ROS-mediated mitochondrial pathway. *Chem-Biol Interact* 199: 129-136.
12. Efferth T (2007) Willmar Schwabe Award 2006: antiplasmodial and antitumor activity of artemisinin - from bench to bedside. *Planta Med* 73: 299-309.
13. Fulda S (2010) Modulation of apoptosis by natural products for cancer therapy. *Planta Med* 76: 1075-1079.
14. Zhang X, Chen LX, Ouyang L, Cheng Y, Liu B (2012) Plant natural compounds: targeting pathways of autophagy as anti-cancer therapeutic agents. *Cell Prolif* 45: 466-476.
15. Newman DJ, Cragg GM (2012) Natural products as sources of new drugs over the 30 years from 1981 to 2010. *J Nat Prod* 75: 311-335.
16. Szeto J, Kaniuk NA, Canadien V, Nisman R, Mizushima N, et al. (2006) ALIS are stress-induced protein storage compartments for substrates of the proteasome and autophagy. *Autophagy* 2: 189-199.
17. Pedro M, Lourenço CF, Cidade H, Kijjoo A, Pinto M, et al. (2006) Effects of natural prenylated flavones in the phenotypical ER (+) MCF-7 and ER (-) MDA-MB-231 human breast cancer cells. *Toxicol Lett* 164: 24-36.
18. Mori S, Sawada T, Okada T, Ohsawa T, Adachi M, et al. (2007) New anti-proliferative agent, MK615, from Japanese apricot "*Prunus mume*" induces striking autophagy in colon cancer cells *in vitro*. *World J Gastroenterol* 13: 6512-6517.
19. Lin HM, Tseng HC, Wang CJ, Chyau CC, Liao KK, et al. (2007) Induction of autophagy and apoptosis by the extract of *Solanum nigrum* Linn in HepG2 cells. *J Agric Food Chem* 55: 3620-3628.
20. Delmulle L, Vanden Berghe T, Keukeleire DD, Vandenabeele P (2008) Treatment of PC-3 and DU145 prostate cancer cells by prenylflavonoids from hop (*Humulus lupulus* L.) induces a caspase-independent form of cell death. *Phytother Res* 22: 197-203.
21. Meschini S, Condello M, Calcabrini A, Marra M, Formisano G, et al. (2008) The plant alkaloid voacamine induces apoptosis-independent autophagic cell death on both sensitive and multidrug resistant human osteosarcoma cells. *Autophagy* 4: 1020-1033.
22. Lin MH, Liu SY, Liu YC (2008) Autophagy induction by a natural ingredient of areca nut. *Autophagy* 4: 967-968.
23. Liu SY, Lin MH, Hsu YR, Shih YY, Chiang WF, et al. (2008) Arecoline and the 30-100 kDa fraction of areca nut extract differentially regulate mTOR and respectively induce apoptosis and autophagy: a pilot study. *J Biomed Sci* 15: 823-831.
24. Yang J, Wu LJ, Tashino SI, Onodera S, Ikejima T (2008) Reactive oxygen species and nitric oxide regulate mitochondria-dependent apoptosis and autophagy in evodiamine-treated human cervix carcinoma HeLa cells. *Free Radic Res* 42: 492-504.
25. Bredholt T, Dimba EA, Hagland HR, Wergeland L, Skavland J, et al. (2009) Camptothecin and khat (*Catha edulis* Forsk.) induced distinct cell death phenotypes involving modulation of c-FLIP<sub>L</sub>, Mcl-1, procaspase-8 and mitochondrial function in acute myeloid leukemia cell lines. *Mol Cancer* 8: 101.
26. King FW, Fong S, Griffin C, Shoemaker M, Staub R, et al. (2009) Timosaponin AIII is preferentially cytotoxic to tumor cells through inhibition of mTOR and induction of ER stress. *PLoS ONE* 4: e7283.
27. Stander A, Marais S, Stivaktas V, Vorster C, Albrecht C, et al. (2009) *In vitro* effects of *Sutherlandia frutescens* water extracts on cell numbers, morphology, cell cycle progression and cell death in a tumorigenic and a non-tumorigenic epithelial breast cell line. *J Ethnopharmacol* 124: 45-60.
28. Juncker T, Schumacher M, Dicato M, Diederich M (2009) UNBS1450 from *Calotropis procera* as a regulator of signaling pathways involved in proliferation and cell death. *Biochem Pharmacol* 78: 1-10.
29. Brunelli E, Pinton G, Bellini P, Minassi A, Appendino G, et al. (2009) Flavonoid-induced autophagy in hormone sensitive breast cancer cells. *Fitoterapia* 80: 327-332.
30. Lei HY, Chang CP (2009) Lectin of Concanavalin A as an anti-hepatoma therapeutic agent. *J Biomed Sci* 16:10.
31. Law BYK, Wang M, Ma DL, Al-Mousa F, Michelangeli F, et al. (2010) Alisol B, a novel inhibitor of the sarcoplasmic/endoplasmic reticulum Ca(2+) ATPase pump, induces autophagy, endoplasmic reticulum stress, and apoptosis. *Mol Cancer Ther* 9: 718-730.
32. Ooi KL, Muhammad TST, Sulaiman SF (2010) Growth arrest and induction of apoptotic and non-apoptotic programmed cell death by *Physalis minima* L. chloroform extract in human ovarian carcinoma Caov-3 cells. *J Ethnopharmacol* 128: 92-99.
33. Carr G, Williams DE, Díaz-Marrero AR, Patrick BO, Bottrill H, et al. (2010) Bafilomycins produced in culture by *Streptomyces* spp. isolated from marine habitats are potent inhibitors of autophagy. *J Nat Prod* 73: 422-427.
34. Thyagarajan A, Jedinak A, Nguyen H, Terry C, Baldrige LA, et al. (2010) Triterpenes from *Ganoderma lucidum* induce autophagy in colon cancer through the inhibition of p38 mitogen-activated kinase (p38 MAPK). *Nutr Cancer* 62: 630-640.

Compound/agent	Species	Cell line	Autophagic markers	Reference
puromycin		HeLa cervix cancer, DU145 prostate cancer, RAW264.7 macrophages	formation of polyubiquitinated defective ribosomal products (DRiPs) ↑	[16]
prenylated flavones		ER(+) and MCF-7 ER(-) MDA-MB-231 breast cancer	autophagic vacuolization monodansylcadaverine staining (=autophagosome-specific dye)	[17]
MK615 extract	<i>Prunus mume</i> (Japanese apricot)	SW 480, CoLo, WiDr Colon Ca	autophagic vacuoles, ATG8 immuno-fluorescence [apoptosis]	[18]
extract	<i>Solanum nigrum</i> L.	HepG2 liver cancer	low doses: autophagic vacuoles, LC-I and LC-II expression ↑, Bcl-2 and Akt expression ↓ high doses: p-JNK and Bax expression ↑ apoptosis	[19]
xanthohumol, isoxanthohumol, 8-prenylnaringenin, 6-prenylnaringenin	<i>Humulus lupulus</i> L.	PC-3, Du 145 prostate cancer	autophagic vacuoles, no caspase-3 activation, no apoptosis	[20]
voacamine	<i>Peschiera fuchsiaeifolia</i>	Multidrug-resistant osteosarcoma	autophagic vacuolization, LC-3 expression and conversion, monodansylcadaverine staining	[21]
proteoglycan extract	areca nut	CE81T/VGH cells	autophagic vacuoles, acidic vesicles, LC3-I cleavage	[22]
30-100 kDa extract	areca nut		autophagic vacuoles, acidic vesicles, phosphorylation of mTOR ↓, LC-I cleavage	[23]
evodiamine	<i>Tetradium spec.</i>	HeLa cervix cancer	monodansylcadaverine staining, Beclin-I expression ↑, LC3 expression ↑, 3-MA(= autophagy inhibitor) decreased cell viability	[24]

organic extract	<i>Catha edulis</i> Forsk.	Leukemia cell lines MOLM-13	morphological features of autophagy apoptosis: McR-1↓, c-FLIPL cleavage ↑, pro-caspase-8 activation ↑; mitochondrial damage	[25]
timasaponin A III	<i>Anemarrhena asphodeloides</i>	various cancer cell lines	mTORC1 inhibition induction of endoplasmic reticulum stress (eIF2alpha phosphorylation ↑; caspase-4 activation↑)→pro-apoptotic pathways	[26]
extract	<i>Sutherlandia frutescens</i>	MCF-7 breast cancer	autophagic vacuolarization hypercondensed chromatin→apoptosis	[27]
UNBS 1450 01 (=derivative of 2-oxovoruscharin 02)	<i>Calotropis procera</i>		sodium pump inhibitor, disorganization of actin cytoskeleton	[28]
isocannflavin B		T47D	autophagic vacuolization, Akt phosphorylation ↓; p21 (Cip21) expression↑	[29]
concanavalin A	Jack bean seeds		autophagy, checken immunomodulation	[30]
alisol B	<i>Alisma orientale (rhizome)</i>	various cancer cell lines	autophagosome flux formation; cell death, G <sub>0</sub> /G <sub>1</sub> cell cycle arrest; activation of CaMKK-AMPK-mTOR pathway; disruption of Ca <sup>2+</sup> homeostasis; binding to SERCA	[31]
chloroform extract	<i>Physalis minima L.</i>	Caov-3	Human ovarian cancer vacuolated morphology; [apoptosis]	[32]
bafilomycin F, G, H, and J	<i>Streptomyces</i> ssp.	MCF-7 breast cancer	microscopy and biochemical assays	[33]
triterpene extract	<i>Ganoderma lucidum</i>	Colon cancer	autophagic vacuoles, Beclin-1↑, LC-3↑; Go/G1 cell cycle arrest; cell death; inhibition of p38 MAPK reduced autophagy	[34]
artesanate	<i>Artemisia annua</i>	MCF-7 breast cancer	endolysosomal and autophagosomal compartments, inhibiting autophagosome turnover and causing perinuclear clustering of autophagosomes, early and late endosomes, and lysosomes.	[9]

**Table 1:** Natural products inducing autophagy in cancer cells.