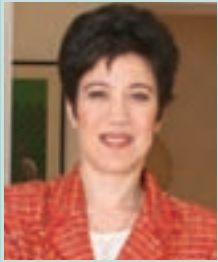


## Autophagy and Colorectal Cancer Therapy



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### Definition and functions of autophagy

The term autophagy is derived from Greek and means 'self-cannibalism'. In the cellular context, it involves degradation of cytoplasmic components by the cell's own lysosomal system. At the initial stages of autophagy, bulk cytoplasm with mitochondria and endoplasmic reticulum are enwrapped in a double-membrane vesicle called an autophagosome. This fuses with lysosomes to form the autolysosome where the final hydrolytic degradation of the contents takes place. Increased autophagy is observed in healthy cells during tissue remodelling and under cellular stress [1]. It is also associated with various pathological conditions such as infection, neurodegenerative diseases and cancer [2,3].

Several functions of autophagy have been identified. Firstly, it is a homeostatic mechanism that relieves the burden of excess protein and old injured organelles. Secondly, recycling of proteins provides amino acids necessary for survival under conditions of nutrient deprivation. Finally, extensive autophagy leads to overwhelming loss of organelles resulting in cell death. The latter is denoted programmed cell death type II to distinguish it from apoptosis. The two processes display large differences in cellular morphology and the molecular pathways that govern them (Table 1). Depending on cellular context and external signals, autophagy can either be required to trigger apoptosis, antagonise apoptosis or occur independently as a back-up cell death mechanism. Exploration of those relationships is fundamental to the development of successful therapeutic strategies.

### Autophagy and colorectal cancer therapy

Many anticancer drugs owe their therapeutic effect to induction of apoptotic cell death [4]. A number of these agents have also been found to induce autophagy. With regards to colorectal cancer, the overwhelming majority of data point to the role of

autophagy in the delay of apoptosis. The mechanism underlying this antagonistic relationship is unclear. One theory proposes that autophagic degradation of damaged mitochondria prevents release of pro-apoptotic factors, such as cytochrome c, by these organelles [5]. From the therapeutic view point, the inhibition of autophagy is likely to bring on the desired therapeutic effect for colorectal cancers. This has been demonstrated by recent studies involving autophagy inhibitors: 3-MA and Bafilomycin A1, which interfere with the formation of autophagosome and autolysosome, respectively. Both agents have been shown to inhibit autophagy induced by bile acid in both normal and cancerous colon, whereby they increased cell death by apoptosis. An opposite, anti-apoptotic, effect was seen following treatment with Rapamycin, which enhances autophagy by blocking the mTor pathway [6].

Promising data came from several studies exploring agents that could enhance the anticancer effect of 5-fluorouracil. 5-FU is a gold standard treatment for colon cancer. It exerts its therapeutic effect by inducing apoptosis. However, alongside this, 5-FU elicits autophagic survival response. Hence, the term autophagy-dependent resistance has been used in the context of 5-FU therapy. Autophagy inhibition by 3-MA was shown to significantly increase the apoptosis-inducing effect of 5-FU in two colon cancer cell lines, HT29 and colon26 [7]. Similar results come from the combination of 5-FU with chloroquine, an antimalarial drug that was shown to have autophagy-inhibiting properties [8]. Another combination therapy involving phytochemicals (I3C) and genistein derived from cruciferous plants and soybeans respectively was shown to inhibit autophagy and promote apoptotic cell death in colon cancer cells. Its advantage lies in the relative lack of toxicity at the pharmacologically effective doses [9].

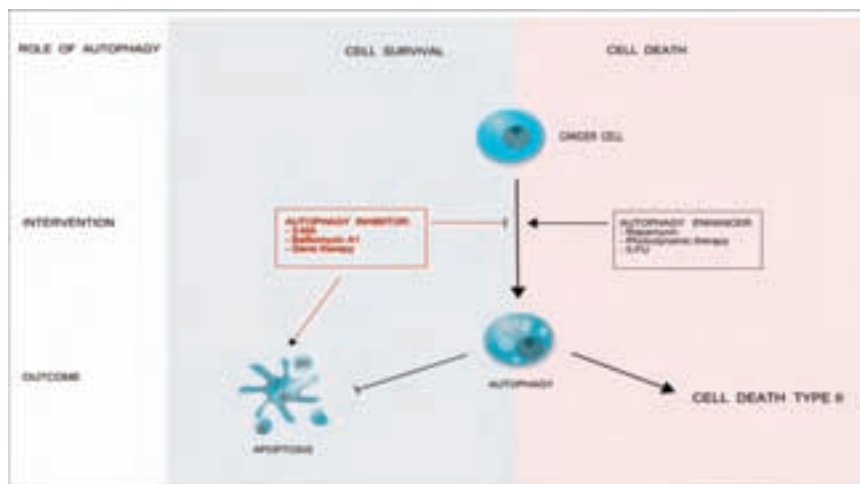


Figure 1:  
Two potential  
autophagy-targeting  
approaches in colon  
cancer therapy.

**Table 1: Comparison of autophagy and apoptosis**

Type of cell death		Autophagy	Apoptosis
Morphological features	Nucleus	Intact	Fragmented
	Plasma membrane	Intact	Blebbing, formation of apoptotic bodies
	Cytoplasm	Multimembrane vacuoles, enlargement of Golgi apparatus	Shrinkage
	Engulfment by phagocytes	no	yes
Trigger		Nutritional deprivation	Intrinsic path: DNA damage, oxidative stress
		Hypoxia	Extrinsic path: ligand activation of death receptor
Regulation	Positive	PI 3-kinase III (Beclin 1)	Intrinsic path: Bax and Bak
		PTEN/inhibition of mTor Ras	Extrinsic path: death receptor activation by TNF, Fas, TRAIL
	Negative	PI 3-kinase/Akt/mTor/ gene expression	Bcl-2, TGF- $\beta$ , P53, IGF-1
Outcome and implications for cancer		Cell survival – favours tumour growth and protects against anticancer therapy	Programmed cell death – suppresses tumour growth, the primary outcome of many anticancer therapies
		Programmed cell death II – supports anticancer therapy	
Inhibitors and their action		3-MA – inhibits autophagosome formation Bafilomycin 1A – inhibits autolysosome formation Chloroquine	
Inducers and their action		Rapamycin – mTor inhibitor 5-FU Photodynamic Therapy (PDT) Triterpenoid saponins Ionizing radiation I3C (cruciferous plants) Genistein (soybean)	5-FU Cetuximab – mAb against EGFR Bevacizumab – mAb against VEGF Oblimersen – antisense to Bcl-2

A separate line of investigation points to the role of autophagy in cell death and suppression of colon cancer. Extract from a mushroom used in traditional Chinese medicine, as well as, a polysaccharide produced by probiotic bacterium *Lactobacillus acidophilus* have both been found to induce autophagic cell death type II in colon cancer [10-12]. There is evidence that 5-FU-induced autophagy can function as a cell death type II mechanism. This was demonstrated in experiments on apoptosis-defective cancers where Bax and Bak proapoptotic factors are knocked-out. In this setting, autophagy acts as an alternative death mechanism [13].

Gene therapy, which is predicted to grow in importance in the near future, can be used to modulate autophagy. Gene manipulations can be achieved by using short-interfering RNA which blocks translation of selected genes to their protein products. Components of the autophagic process, such as Atg7, present potential targets for gene silencing [2]. However, in the present day, gene therapy is still considered impractical in the clinical setting. Nonetheless, the future may bring technologies capable of silencing autophagy-related genes safely and effectively.

Collectively these data suggest a paradoxical role of autophagy as a target for adjuvant therapy. On one hand it allows cell

survival against cytotoxicity of anticancer agents in apoptosis-competent cancer. On the other, it acts as a backup cell death mechanism in apoptosis-defective cancer. Several ways of manipulating autophagy have been developed allowing for both inhibition and enhancement of the process (Figure 1). Before they become part of routine treatment two issues need addressing. Firstly, accurate ways of determining a patients' unique cancer biology must be developed to guide choice of patient-tailored, either autophagy-inducing or inhibiting, approach. Secondly, the agents already found effective in in vivo and in vitro studies require further testing to prove their utility in a clinical setting. ■

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