



THERAPEUTIC HORIZONS OF AUTOPHAGY AND APOPTOSIS IN COLORECTAL CANCER: A TRANSLATIONAL PERSPECTIVE FROM MOLECULES TO MEDICINE

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ABSTRACT

Autophagy and apoptosis are fundamental cellular processes that profoundly influence colorectal cancer (CRC) development, progression, and response to therapy. As our molecular understanding of these pathways has deepened, so too have efforts to exploit them for therapeutic benefit. This review provides a comprehensive overview of autophagy and apoptosis mechanisms in CRC, highlighting key regulatory molecules and crosstalk between the pathways, and examines current and emerging therapeutic strategies that target these processes – from bench to bedside. We discuss how autophagy can act as both a tumor suppressor and a resistance mechanism in CRC, the frequent dysregulation of apoptosis pathways (e.g., p53 mutations, Bcl-2 family overexpression) that allows colorectal tumor cells to evade cell death, and how these insights are guiding translational research. Therapeutic agents that modulate autophagy (such as chloroquine derivatives, mTOR inhibitors, and novel autophagy inhibitors) and those that promote apoptosis (including BH3 mimetics targeting Bcl-2 family proteins, death receptor agonists, and p53 reactivators) are critically reviewed. Preclinical studies demonstrate that inhibiting autophagy can sensitize CRC cells to chemotherapy-induced apoptosis, and conversely, promoting autophagic flux under certain conditions can drive type II cell death. The translational pipeline features several combination approaches – for example, trials of hydroxychloroquine with chemotherapy to overcome treatment resistance, and BH3 mimetic drugs to restore apoptotic potential in refractory CRC. We also explore how autophagy and apoptosis modulation intersect with other modalities like immunotherapy (e.g., autophagy influences immunogenic cell death and antigen presentation) and targeted therapies (e.g., oncogenic signaling inhibitors may induce compensatory autophagy). By integrating preclinical data with clinical trial results, we outline the “therapeutic horizons” wherein manipulating cell death pathways could improve outcomes for CRC patients. This includes a look at precision medicine approaches: selecting patients based on biomarkers such as Bcl-2 family expression profiles or autophagy activity for tailored therapies. In summary, the translation of molecular insights in autophagy and apoptosis into clinical interventions is underway, holding promise for new treatments that can effectively eliminate colorectal cancer cells by tipping the balance from survival to death.

Key words: CRC, Autophagy, apoptosis, Colorectal Cancer

INTRODUCTION

Colorectal cancer is driven by a cascade of genetic and epigenetic changes that enable unchecked cell proliferation and survival. Two central biological processes often perturbed in CRC are autophagy – the self-cannibalization mechanism for cellular recycling and quality control – and apoptosis – the programmed cell death pathway. In normal physiology, these pathways serve as a yin and yang of cellular fate: autophagy

generally promotes survival under stress by eliminating damaged components, whereas apoptosis irreversibly commits a cell to death when damage or signals exceed a threshold. In cancer cells, however, both autophagy and apoptosis pathways are subverted: autophagy can be co-opted to support cancer cell survival, and apoptosis is frequently disabled to allow tumor cells to evade destruction.

In CRC, autophagy plays complex roles. During the early stages of tumorigenesis, functional autophagy can suppress tumor initiation by maintaining genomic stability and preventing accumulation of reactive oxygen species and protein aggregates. Indeed, loss-of-function mutations or reduced expression in certain autophagy-related genes (ATGs) have been linked to increased colorectal cancer risk. For example, monoallelic deletion of the autophagy gene *BECN1* (encoding Beclin-1) is observed in some cancers, and in CRC, low Beclin-1 expression has been associated with tumor progression. However, once a colorectal tumor is established, autophagy often becomes a survival mechanism that helps cancer cells adapt to metabolic stress and resist therapies. Cytotoxic treatments like chemotherapy and radiation can induce autophagy in CRC cells, which may act as a shield against apoptosis, thereby contributing to treatment resistance. This “Janus-faced” nature of autophagy – tumor-suppressive in origin but tumor-promoting in later stages – makes it a challenge and an opportunity for therapy.

Apoptosis evasion is a hallmark of colorectal cancer. Under normal conditions, cells that acquire oncogenic mutations or suffer severe DNA damage would undergo apoptosis, often through the intrinsic (mitochondrial) pathway controlled by p53 and Bcl-2 family proteins. However, p53 is mutated in ~50% of CRC cases, impairing the cell's ability to trigger apoptosis in response to DNA damage. Furthermore, many CRCs show dysregulation of the Bcl-2 family: some tumors upregulate anti-apoptotic members (such as Bcl-X_L, Mcl-1, or Bcl-2 itself) or downregulate pro-apoptotic members (Bax, Bak, or BH3-only proteins like Bid, Bim). For instance, microsatellite-unstable CRCs frequently acquire frameshift mutations in the pro-apoptotic gene *BAX*, rendering cells less responsive to apoptotic signals. The net result is that colorectal cancer cells become highly apoptosis-resistant –

they can tolerate genotoxic stress that would kill a normal cell. This resistance underlies not only cancer development (allowing survival of aberrant cells) but also treatment failure, as many chemotherapy drugs rely on apoptosis to eliminate cancer cells.

Given the significance of these pathways, there has been intense interest in developing therapies that reactivate apoptosis or modulate autophagy in CRC. The therapeutic rationale can be summarized as follows: (1) Induce apoptosis – push cancer cells over the edge by targeting the apoptotic blockades (e.g., inhibit Bcl-2/Bcl-X_L or mimic pro-apoptotic signals) so that tumor cells can no longer escape programmed death; (2) Manipulate autophagy – either inhibit autophagy to prevent cancer cells from surviving under therapy stress, or in some contexts, hyperactivate autophagy to drive cells into autophagic cell death. Both strategies have shown promise in preclinical models, and an array of drugs affecting these pathways have entered clinical trials for CRC or are in development. In this review, we traverse the journey “from molecules to medicine,” starting with the mechanistic underpinnings of autophagy and apoptosis in colorectal cancer, and moving to current translational and clinical efforts. We will first provide an overview of the molecular pathways of autophagy and apoptosis in CRC, including key regulators and how they interact. Next, we will examine therapeutic agents and targets that modulate these pathways: for autophagy (e.g., chloroquine, ULK1 inhibitors, dietary modulation) and for apoptosis (e.g., BH3 mimetics targeting Bcl-2 family, death receptor agonists like TRAIL, and emerging p53-targeted treatments). Finally, we will discuss how preclinical findings are being applied in clinical trials, highlighting successes, setbacks, and what lies ahead – the **therapeutic horizons** – for leveraging autophagy and apoptosis in treating colorectal cancer.

Table 1. Key Regulators of Autophagy and Apoptosis in Colorectal Cancer

Molecule	Role in Autophagy	Role in Apoptosis	Therapeutic Implication
Beclin-1	Initiates autophagy via PI3K complex	Cleaved during apoptosis to promote death	Target for modulating autophagy flux
Bcl-2	Inhibits Beclin-1 and autophagy	Anti-apoptotic, binds Bax/Bak	Target for BH3 mimetics
p53	Regulates autophagy genes under stress	Activates Bax, Puma, Noxa for apoptosis	p53 activators under trial
LC3-II	Autophagosome marker	N/A	Used to monitor autophagy activity
Mcl-1	Maintains mitochondrial integrity under stress	Anti-apoptotic protein	Target for selective Mcl-1 inhibitors

Table 2. Therapeutic Agents Targeting Autophagy and Apoptosis in CRC

Agent/Class	Target/Mechanism	Current Status
Hydroxychloroquine	Blocks autophagosome-lysosome fusion	In Phase II trials
BH3 mimetics (e.g., navitoclax)	Inhibit Bcl-2/Bcl-X _L	Preclinical/early clinical
Mcl-1 inhibitors	Disrupt Mcl-1 anti-apoptotic function	Preclinical/early clinical
ULK1 inhibitors	Block autophagy initiation	Preclinical
TRAIL agonists	Activate death receptors DR4/5	Limited clinical response; under combination trials

Table 3. Crosstalk Mechanisms Between Autophagy and Apoptosis

Mechanism	Description	Implication
Beclin-1 cleavage	Caspase cleavage promotes apoptosis	Loss of autophagy promotes cell death
Bcl-2 binding	Inhibits both Beclin-1 and Bax	A key checkpoint between survival and death
Caspase-8 degradation	Autophagy may degrade apoptotic initiators	Potential escape from extrinsic apoptosis
BH3-only proteins	Displace Beclin-1 from Bcl-2	Activate both pathways under stress

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Mechanisms of Autophagy in Colorectal Cancer

Autophagy-Apoptosis Crosstalk

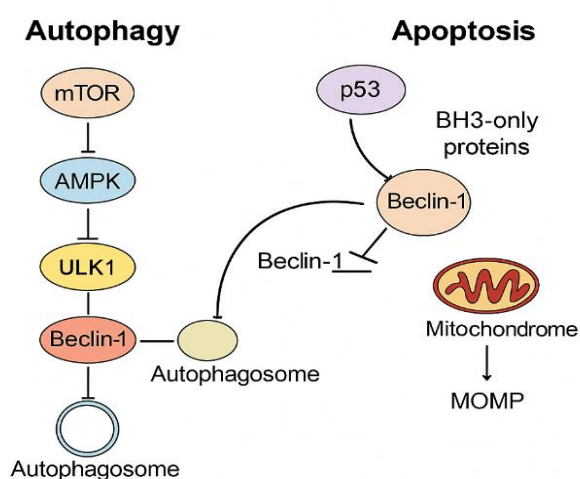


Figure 1. Crosstalk between autophagy and apoptosis in colorectal cancer. Autophagy is triggered by reduced growth factors, activating AMPK and ULK1 to form autophagosomes. Crosstalk between Beclin-1 and Bcl-2 family proteins influences whether the cell survives or undergoes apoptosis.

Autophagy Signaling Pathways: Macroautophagy (hereafter autophagy) is orchestrated by a set of ATG proteins and complexes that regulate the formation of double-membraned autophagosomes and their fusion with lysosomes. Nutrient-sensing pathways govern the induction of autophagy. A central player is the kinase mTOR (mechanistic Target of Rapamycin), which, when active (in nutrient-rich conditions), phosphorylates and inhibits ULK1/2, the initiator complex of autophagy. Under energy stress or growth factor deprivation – conditions relevant to CRC tumors experiencing intermittent nutrient supply or areas of poor vascularization – AMPK is activated and mTOR is inhibited, thereby unleashing ULK1 and initiating autophagosome formation. This involves the Beclin-1/Vps34 complex (class III PI3K complex) that generates phosphatidylinositol-3-phosphate and recruits downstream ATG proteins to nucleate the autophagosome membrane. Two ubiquitin-like conjugation systems (ATG5–ATG12 and LC3–phosphatidylethanolamine) are required for autophagosome expansion and completion. Beclin-1 is a critical scaffold in the autophagy initiation complex, and as noted, is subject to regulation by various binding partners (e.g., Bcl-2, UVRAG, Ambra1) and modifications.

In colorectal cancer cells, these autophagy pathways operate within a cancer-specific context. Studies have shown that oncogenic signaling in CRC, such as activated **KRAS** or **BRAF**, can upregulate basal autophagy. KRAS^{mut} CRC cells often exhibit elevated autophagy flux, which is thought to help meet their metabolic demands and maintain mitochondrial function. This has been observed as increased LC3-II levels and autophagosome count in KRAS-driven CRC models compared to wild-type KRAS counterparts. Oncogenic PI3K/Akt signaling, conversely, tends to suppress autophagy via mTOR; thus, CRC tumors with PI3K activation (common through PIK3CA mutations) might have lower autophagy unless counteracted by other signals. The tumor microenvironment in CRC (which often includes regions of **hypoxia**) is another autophagy trigger: Hypoxia stabilizes HIF-1 α , which can induce BNIP3/BNIP3L (NIX), BH3-only proteins that displace Bcl-2 from Beclin-1 and activate autophagy. Many CRCs, especially high-grade tumors, experience hypoxic stress and accordingly show hypoxia-induced autophagy, helping cells survive low oxygen and nutrient conditions.

Autophagy's Role in Tumor Initiation vs Progression: As briefly mentioned, autophagy can suppress the initiation of CRC. Mice with systemic heterozygous deletion of *Beclin1* have higher incidence of spontaneous tumors, and in models of colitis-associated CRC, autophagy deficiency (e.g., *Atg5* or *Atg7* knockout in

intestinal epithelium) leads to more inflammation, dysplasia, and cancer, partly due to unresolved oxidative damage and an impaired barrier against gut microbiota. Clinically, some early lesions like adenomas show lower expression of autophagy proteins compared to normal tissue. However, once a tumor is formed, autophagy can assist cancer cell survival. In vitro, CRC cell lines often upregulate autophagy in response to chemotherapy as a defense mechanism. For example, treating CRC cells with 5-FU leads to increased LC3-II and autophagosome formation; when autophagy is blocked by 3-methyladenine or chloroquine, those cells undergo more apoptosis in response to 5-FU. This suggests that autophagy is helping the cells cope with drug-induced stress, and its inhibition makes them less capable of resisting apoptosis.

Moreover, autophagy contributes to the maintenance of cancer stem cells and therapy-resistant persisted cells in CRC. There is evidence that a subset of colon cancer stem-like cells relies on autophagy for maintaining their quiescence and survival under hostile conditions (like chemotherapy). Inhibiting autophagy can preferentially impact these cells, which otherwise could regenerate the tumor after treatment. Thus, autophagy is implicated in minimal residual disease and recurrence.

Genetic Alterations in Autophagy Components: Unlike some cancers (e.g., pancreatic cancer) where mutations in autophagy genes are frequent, in CRC it's more about expression changes and functional regulation than hard mutations. That said, a fraction of CRCs harbor mutations in autophagy regulators: e.g., loss-of-function mutations in *UVRAG* (a Beclin-1 partner) and *ATG5* have been reported in some colorectal tumors. *UVRAG* is located in a region (11q13) that can undergo allelic loss; reduced *UVRAG* impairs autophagosome maturation and has been linked to chromosomal instability in colon cancer cells. Likewise, frameshift mutations in ATG genes with microsatellites (like *ATG2B*, *ATG5*) occur in MSI-high CRCs. The functional impact is still being elucidated, but generally loss of these ATGs could tip the scales towards genome instability and cancer progression (as autophagy can no longer perform quality control).

On the other end, overexpression of certain pro-autophagy genes is seen. *ATG10*, for instance, was found upregulated in CRC tissues, correlating with metastasis and invasion. High *ATG10* might facilitate an increased autophagic capacity that tumor cells exploit during detachment, circulation, and colonization of metastases. Another gene, Beclin-1, as discussed, can be either under- or overexpressed. Koukourakis *et al.* (2010) showed that about 21% of CRC had “extensive overexpression” of Beclin-1, often in more aggressive, hypoxic tumors. This might indicate those tumors are heavily using autophagy to survive microenvironmental stress.

Consequences of Autophagy Modulation: For therapy, the key takeaway is that **inhibiting autophagy in established CRC is generally pro-death and anti-tumor**, especially when combined with other treatments. Autophagy inhibition deprives cancer cells of a crucial stress tolerance mechanism. For example, CQ or HCQ treatment in CRC cell lines can trigger accumulation of dysfunctional mitochondria and heightened ROS, leading to DNA damage and activation of intrinsic apoptosis (if apoptosis pathways are intact). It can also prevent the turnover of proteins that, when accumulated, may be toxic to cells. In one study, autophagy inhibition caused buildup of the pro-apoptotic protein Noxa, which then contributed to killing CRC cells in combination with oxaliplatin.

Conversely, there might be scenarios (e.g., certain oncogene-addicted contexts) where *inducing* autophagy could be leveraged to kill CRC cells – essentially pushing them into autophagic cell death. Some researchers have experimented with using mTOR inhibitors (like rapamycin or everolimus) to drive autophagy in CRC, but this alone rarely kills cancer cells outright; instead, it might slow growth. However, combining extreme autophagy induction with apoptosis induction could synergize – for instance, a TRAIL agonist (apoptosis inducer) combined with an mTOR activator (to maximize autophagy) was reported to overcome resistance in a CRC model by simultaneously engaging both death pathways.

In summary, autophagy in CRC is a double-edged sword: protective early, but then co-opted by tumors later. Therapeutically, blocking autophagy tends to make established cancers more vulnerable, which underlies ongoing trials of autophagy inhibitors in CRC (covered later).

Mechanisms of Apoptosis in Colorectal Cancer

Intrinsic and Extrinsic Apoptosis Pathways: Apoptosis can be initiated via two main routes – the intrinsic (mitochondrial) pathway and the extrinsic (death receptor) pathway – both of which ultimately activate caspases that dismantle the cell. The intrinsic pathway is tightly regulated by the **Bcl-2 family**. Pro-apoptotic members (Bax, Bak) form pores in the mitochondrial membrane when activated, releasing cytochrome c to trigger caspase-9 and the downstream executioner caspases (3, 7). Anti-apoptotic members (Bcl-2, Bcl-X_L, Mcl-1, etc.) prevent this by binding and sequestering pro-apoptotic members or their activator BH3-only proteins (like Bim, Bid, Puma, Noxa). In healthy cells, there is a delicate balance in this family. In CRC cells, this balance is often skewed toward survival. For example, **Bax is a crucial pro-apoptotic protein that is inactivated in a subset of CRCs**: in microsatellite-unstable tumors, a common frameshift mutation in the BAX gene (which contains a G8 microsatellite in its coding region) leads to a truncated,

nonfunctional Bax protein. Approximately half of MSI-high CRCs have such BAX mutations, contributing to these tumors' notorious resistance to apoptosis (which may be one reason MSI-high metastatic CRC historically responded poorly to chemotherapy, although they respond well to immunotherapy for other reasons).

In microsatellite-stable CRCs, Bax is usually intact, but the pathways that activate it may be impaired. For instance, p53, a major transcriptional activator of pro-apoptotic genes (including BAX, PUMA), is mutated in roughly 50% of CRCs as noted. Loss of p53 function removes a key apoptosis trigger in response to DNA damage or oncogene-induced stress. Therefore, many CRC cells lack the ability to upregulate BH3-only proteins like PUMA and NOXA after chemotherapy, blunting the apoptotic response. Even if p53 is wild-type (as in ~50% of cases), downstream components can be altered: some CRCs overexpress inhibitors of apoptosis proteins (IAPs) that can degrade active caspases, or have upregulated survival pathways (like NF- κ B) that increase expression of anti-apoptotic Bcl-2 family members.

The extrinsic pathway involves ligation of death receptors (such as FAS/CD95 or TRAIL receptors DR4/DR5) by their ligands, leading to formation of the death-inducing signaling complex and activation of caspase-8. CRC cells can be resistant to extrinsic apoptosis as well – common reasons include downregulation or mutation of the receptors (some CRC cell lines have low DR4/DR5 expression), and increased FLIP (FLICE-like inhibitory protein) which inhibits caspase-8 activation. Despite these, TRAIL (TNF-related apoptosis-inducing ligand) was found to induce apoptosis in many CRC cell lines preferentially over normal cells, which spurred development of TRAIL agonists. Unfortunately, in clinical trials, recombinant TRAIL or agonistic antibodies against DR4/5 showed limited efficacy in unselected patients, partly because many tumors had intrinsic or acquired resistance. Mechanisms of resistance include the aforementioned loss of receptors on the cell surface and activation of pro-survival pathways under treatment pressure.

Aberrations in Bcl-2 Family in CRC: Unlike some lymphomas where BCL2 gene is translocated and massively overexpressed, in CRC the regulation is more nuanced. Immunohistochemical studies have unexpectedly shown that Bcl-2 protein is present in a subset of colon tumors and is associated with *lower* grade and improved survival. As noted earlier, this counterintuitive scenario arises because Bcl-2 tends to be expressed in early adenomas and in differentiated tumor cells, whereas poorly differentiated or advanced tumors lose Bcl-2 but often gain Bcl-X_L and Mcl-1. Bcl-X_L (encoded by BCL2L1) is a frequently upregulated anti-apoptotic protein in CRC and

is thought to be a key factor in chemotherapy resistance. For example, one study found that creating a 5-FU-resistant HCT116 CRC cell line led to a dramatic increase in Bcl-X_L expression and concomitant decrease in Mcl-1. Bcl-X_L upregulation conferred a survival advantage, as silencing Bcl-X_L in those cells re-sensitized them to 5-FU-induced death. Mcl-1, on the other hand, is often

elevated in KRAS- or BRAF-mutant CRC cells. BRAF^{V600E} mutant colon cancers have been shown to depend on Mcl-1; in vitro, inhibiting BRAF with a MEK inhibitor caused these cells to upregulate Mcl-1 as a resistance mechanism, but adding an Mcl-1 inhibitor restored cell death.

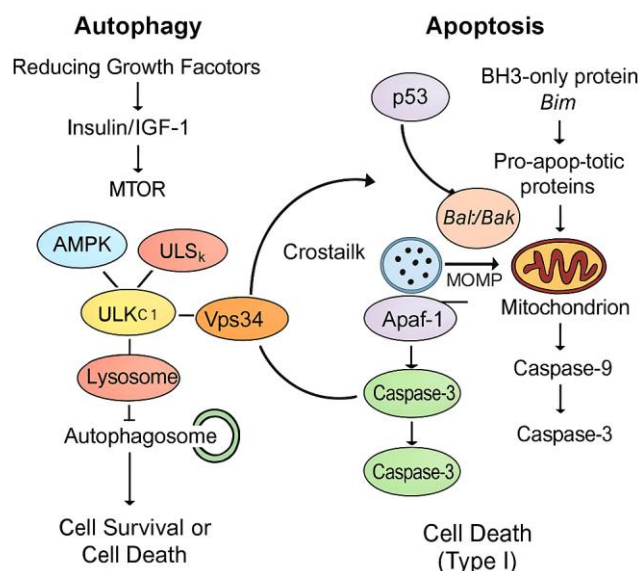


Figure 2. Molecular convergence of autophagy and apoptosis pathways at key regulatory points such as Beclin-1/Bcl-2 and caspase activation in CRC cells. This integration determines cellular fate under stress or therapy.

Pro-apoptotic members can also be altered: aside from Bax, another example is **Bim (encoded by BCL2L11)**, a potent BH3-only protein that activates Bax/Bak. Some CRC cells, especially those with activated EGFR/RAS/MAPK signaling, exhibit low Bim due to constitutive ERK activation that leads to Bim degradation. Low Bim levels correlate with resistance to EGFR inhibitors in CRC (since the apoptosis execution is blunted). In such cases, combining BH3 mimetics or proteasome inhibitors (to boost Bim) with targeted therapy has been suggested.

Implications for Therapy: The molecular map of apoptosis in CRC highlights several targets for therapeutic intervention: (1) **Reactivating p53** – for p53-wildtype tumors, one could use MDM2 inhibitors (e.g., nutlins) to enhance p53's pro-apoptotic function; for p53-mutant tumors, newer agents like APR-246 (which refolds certain p53 mutants) or gene therapy approaches could restore some apoptotic potential. (2) **Bcl-2 family inhibition** – BH3 mimetic drugs like **navitoclax (ABT-263)** target Bcl-2 and Bcl-X_L, and **venetoclax (ABT-199)** targets Bcl-2 specifically. While venetoclax is less relevant for CRC (since Bcl-2 itself is not a primary driver in most CRCs), navitoclax or Bcl-X_L-specific agents (like A-1331852)

are of high interest. There is also S63845, a selective Mcl-1 inhibitor, showing preclinical efficacy in solid tumors. However, in practice, single-agent BH3 mimetics have shown limited effect in solid tumors so far – likely because cancer cells often have redundancy in survival signals or because these drugs trigger cytostatic effects via autophagy as mentioned. Trials are ongoing combining BH3 mimetics with other drugs in solid tumors, including CRC (e.g., navitoclax with MEK inhibitors for KRAS-mutant tumors, given preclinical synergy). (3) **Death receptor agonists** – second-generation TRAIL receptor agonists and DR5-targeted antibodies or ligand traps are under investigation, some conjugated with other immune stimulators to enhance potency. Additionally, **IBC** (immune agonist) approaches like CD3×DR5 bispecific antibodies aim to recruit T-cells to kill DR5-expressing CRC cells, thereby utilizing extrinsic apoptosis via immunological means.

Crosstalk Between Autophagy and Apoptosis: The autophagy and apoptosis pathways do not operate in isolation; they influence each other significantly. We have covered one major node of crosstalk: Bcl-2 family proteins interact with Beclin-1 and other autophagy proteins, thereby linking the decision between survival (autophagy)

and death (apoptosis). Another point of intersection is via substrates of autophagy that are related to apoptosis. For instance, active caspase-8 (an apoptosis initiator) can be degraded by autophagy under certain conditions, dampening extrinsic apoptosis. Conversely, during apoptosis, caspases can cleave some autophagy proteins (such as Beclin-1 or ATG5), converting them into pro-apoptotic fragments – a feed-forward mechanism to ensure the cell commits to death. Thus, when designing therapies, one must consider these interactions: blocking autophagy may remove a brake on apoptosis (as when chloroquine heightened 5-FU cytotoxicity by allowing more apoptosis), whereas hyperactivating apoptosis might automatically shut off autophagy as the cell undergoes demolition.

To comprehensively kill CRC cells, combination approaches that address both pathways may be most effective. In the next section, we discuss how these insights have fueled various therapeutic strategies and what evidence we have so far from translational research.

Therapeutic Strategies Targeting Autophagy and Apoptosis in CRC

Autophagy-Targeted Therapies

Autophagy Inhibition – Hydroxychloroquine and beyond:

The most clinically advanced strategy to target autophagy in cancer is the use of lysosomal inhibitors like chloroquine (CQ) or hydroxychloroquine (HCQ). These agents raise the lysosomal pH and prevent autophagosomes from degrading their cargo, effectively stalling autophagy at the lysosome fusion step. In CRC, as discussed, blocking autophagy can increase cancer cell sensitivity to various stresses. A landmark demonstration in vitro was that using 3-methyladenine (an early-stage autophagy inhibitor) or CQ enhanced 5-FU-induced apoptosis in colon cancer cells. This provided rationale for combining autophagy inhibitors with chemotherapy. Clinically, HCQ has been tested in combination with multiple regimens:

- A **Phase I trial** tested escalating doses of HCQ with a fixed regimen of capecitabine and oxaliplatin in patients with advanced solid tumors (including CRC) and found it was safe to use HCQ up to high doses that achieve autophagy inhibition in peripheral blood mononuclear cells. This set the stage for Phase II trials.
- One **Phase II trial in metastatic CRC** (single-arm) combined HCQ with mFOLFOX6 (5-FU/leucovorin/oxaliplatin) and bevacizumab as first-line therapy. Although a clear efficacy signal was hard to discern without a control arm, the study did report that the addition of HCQ did not significantly worsen toxicity and that a subset of patients had encouraging responses. Correlative studies showed

increased LC3-II and p62 in patient PBMCs, indicating effective autophagy inhibition.

- Another trial in **refractory mCRC** randomized patients to HCQ + vorinostat (an HDAC inhibitor) versus the multikinase inhibitor regorafenib (standard of care in refractory CRC). The combo aimed to both inhibit autophagy (HCQ) and induce cancer cell stress (vorinostat can induce DNA damage and also upregulate death receptors). The results suggested that HCQ + vorinostat had comparable outcomes to regorafenib, and interestingly, analysis of patient T-cells showed increased activation in the HCQ+vorinostat arm, hinting at an immunomodulatory benefit possibly via autophagy inhibition (since autophagy in tumor cells can lead to immunosuppressive adenosine accumulation, etc.). This trial establishes proof-of-concept that autophagy modulation is a viable strategy in CRC patients.

Beyond HCQ, new autophagy inhibitors are being developed to more specifically target autophagy components. Examples include:

- **ULK1 inhibitors:** ULK1 is the initiating kinase of autophagy. Small molecules like ULK-101 are in preclinical testing; inhibiting ULK1 would stop autophagosome formation at the onset. If these can be made potent and selective, they could serve as alternatives to HCQ with potentially fewer off-target effects than affecting the entire lysosome.
- **VPS34 inhibitors:** VPS34 (PI3K class III) is a core part of the Beclin-1 complex. Inhibitors like SAR405 and PIK-III have shown potent autophagy blockage in cells. One challenge is that VPS34 also has roles in endosomal trafficking, so systemic inhibition might have broad effects. Nonetheless, these are being explored in cancer models.
- **ATG4B inhibitors:** ATG4B is a cysteine protease that processes LC3, essential for autophagosome formation. Targeting ATG4B could prevent LC3 recycling. Some early compounds and peptidomimetics have been described, showing antitumor activity in CRC xenografts by hampering autophagy.

At present, HCQ remains the only autophagy inhibitor used in the clinic, but these novel agents may enter trials in coming years. The ideal scenario for autophagy inhibition might be in combination with therapies that induce cytotoxic stress, such as chemotherapy, radiation, or targeted therapies that cells typically resist via autophagy. For instance, EGFR inhibitors in RAS wild-type CRC often lead to autophagy upregulation as a

resistance mechanism; adding autophagy inhibition might enhance their efficacy (this is being tested preclinically).

Autophagy Induction – A Double-Edged Approach: On the flip side, some strategies involve inducing autophagy to kill cancer cells. One such concept is “**autophagy overdrive.**” Certain natural compounds or drugs can hyper-activate autophagy to such an extent that it becomes nonselective and leads to cell death. An example is high-dose trehalose (a sugar that can activate TFEB and autophagy) which was shown to induce cell death in colon cancer models via excessive autophagy. However, these approaches are not as well-validated as autophagy inhibition, and there’s a fine line between autophagy helping cells survive versus causing death.

One context where autophagy induction might be beneficial is immunotherapy. Some forms of immunogenic cell death require autophagy for optimal immune activation (e.g., autophagy can facilitate release of ATP from dying cells as a “find me” signal for immune cells). There is research into combining autophagy inducers with immune checkpoint blockade to see if it improves antigen presentation or immune cell infiltration in CRC (particularly relevant for immunotherapy-resistant microsatellite-stable CRC).

Dietary and Lifestyle Interventions: Interestingly, not all autophagy modulation needs to come from drugs; dietary interventions like caloric restriction or intermittent fasting can induce autophagy and potentially influence therapy outcomes. While fasting mainly was discussed in context of sensitizing cells (which might be via autophagy induction followed by apoptosis), some propose that cyclic fasting could also “flush out” unhealthy cells via autophagy-mediated cell death. Clinical studies are exploring ketogenic diets or fasting-mimicking diets in cancer patients, which may create metabolic states that affect autophagy in tumors. These approaches are adjuncts and require more evidence but represent a holistic way to target cancer cell metabolism and catabolic processes.

Apoptosis-Targeted Therapies

BH3 Mimetics: The development of BH3 mimetic drugs has been a breakthrough in targeting apoptosis pathways. By design, these small molecules mimic the BH3 domain of pro-apoptotic proteins and bind to the hydrophobic groove of anti-apoptotic Bcl-2 family members, displacing pro-apoptotic partners. **Venetoclax (ABT-199)** is FDA-approved for CLL and AML, targeting Bcl-2 selectively. In CRC, venetoclax alone is unlikely to be sufficient because, as noted, Bcl-2 is not the dominant survival factor in most cases. However, studies have found that a subset of CRC cell lines (especially those with low Mcl-1 and Bcl-X_L) do undergo apoptosis with venetoclax. This suggests a potential niche: for example, a CRC that is genomically stable, p53 wild-type, and has a high Bcl-

2/Bax ratio might respond to Bcl-2 inhibition. There have been case reports of venetoclax showing activity in patients with chemo-refractory CRC when combined with other agents.

Navitoclax (ABT-263), which inhibits Bcl-2, Bcl-X_L, and Bcl-w, was tested in some solid tumor trials, but dose-limiting thrombocytopenia (from Bcl-X_L inhibition in platelets) was problematic. Dosing schedules and combinations are being optimized. For CRC, navitoclax combined with irinotecan was tried in a Phase I trial; a few patients had stable disease, but the combination’s efficacy was not clearly superior. More promising is perhaps **targeting Bcl-X_L specifically** since we know Bcl-X_L is a big player in CRC chemoresistance. A-1331852 is a preclinical tool compound that showed potent antitumor effect in mice bearing 5-FU-resistant CRC xenografts, causing tumor regression with tolerable platelet toxicity due to controlled dosing. A safer Bcl-X_L-targeting strategy is under research – one idea is PROTACs (proteolysis-targeting chimeras) that degrade Bcl-X_L specifically in cancer cells (sparing platelets which lack the delivery mechanism). Alternatively, using a Bcl-X_L inhibitor *transiently* during chemotherapy cycles might minimize toxicity yet augment apoptosis in tumors at the critical time.

Mcl-1 Inhibitors: Mcl-1 is an attractive target because many cancers (including subsets of CRC) are “addicted” to Mcl-1 for survival, and Mcl-1 has a short half-life, making it less present in normal long-lived cells like platelets (so inhibitors might be less acutely toxic than Bcl-X_L inhibitors). Several Mcl-1 inhibitors have entered clinical trials (e.g., AMG 176, AZD5991) mainly in leukemia and myeloma patients. For solid tumors, no results yet show dramatic efficacy, but preclinical data suggest combining Mcl-1 inhibitors with other agents (e.g., with Bcl-X_L or Bcl-2 inhibitors for dual blockade, or with MEK inhibitors in RAS-driven cancers) might be needed. For CRC, one scenario could be in BRAF-mutant CRC: a study found that a MEK inhibitor plus an Mcl-1 inhibitor synergistically induced apoptosis in BRAF-mutant CRC cells by simultaneously blocking Mcl-1 and the feedback induction of BIM. Such combinations may enter trials given the dire need for effective treatments in BRAF-mutant CRC (aside from the newly approved BRAF/EGFR inhibitor combo).

Death Receptor Agonists (TRAIL and others): The concept of using the body’s extrinsic apoptosis pathway to kill cancer was very appealing because of TRAIL’s tumor-selectivity (it kills many cancer cells but not most normal cells). Clinical versions of TRAIL (like dulanermin) and agonist antibodies (like mapatumumab for DR4, tigatuzumab for DR5) unfortunately did not show significant activity in phase II trials in CRC patients, either

as monotherapy or combined with chemotherapy. Primary or acquired resistance was common. However, research continues especially in the form of combination therapies – for example, **sensitizing agents** that upregulate death receptors or downregulate FLIP in tumors could make TRAIL agonists effective. Some such agents include HDAC inhibitors (which increase DR5 expression) or proteasome inhibitors (which can lower FLIP and increase pro-apoptotic Noxa). One preclinical study showed that combining the herbal derivative parthenolide with TRAIL significantly increased apoptosis in CRC cells by upregulating DR5 and caspase activation. Another interesting approach is using **nanoparticle delivery** of TRAIL or DR5 agonists along with other drugs to tumor sites, achieving higher local concentrations.

Recently, there is interest in **DR5 agonist antibodies in combination with immune checkpoint inhibitors or chemotherapy**. The idea is that while DR5 agonists alone didn't shrink tumors, they might induce enough cancer cell apoptosis to release antigens and prime an immune response, which checkpoint inhibitors can then amplify – effectively turning a “cold” tumor “hot.” Some trials combining TRAIL pathway agents with PD-1 inhibitors are in early phases.

p53-Targeting Therapies: Since p53 is such a central hub for apoptosis, restoring its function in p53-mutant CRC could be game-changing. A compound called **APR-246 (eprenetapopt)**, which refolds certain mutant p53 to a wild-type conformation, has shown some activity in hematologic malignancies and is being tested in solid tumors. If a CRC has a common p53 mutation like R175H, APR-246 might help trigger apoptosis in response to therapy by reviving p53's transcription of pro-apoptotic genes. Another approach is **gene therapy** – e.g., adenoviral delivery of p53 (such as the product Ad-p53, or Advexin) which was tried in some cancers. Those approaches have had limited success historically, but new vectors and possibly lipid nanoparticle mRNA technology might be revisited.

Caspase Activation and IAP Antagonists: Some therapies aim downstream at the caspases themselves. While you cannot directly “force” a caspase to activate without upstream signals, what you can do is remove inhibitors. Inhibitor of apoptosis proteins (IAPs), such as XIAP, cIAP1/2, bind and inhibit caspases. **Smac mimetics** are drugs that mimic the endogenous protein Smac/DIABLO, which neutralizes IAPs. Smac mimetics (e.g., birinapant, LCL161) have been tested in solid tumor trials. In CRC models, smac mimetics can synergize with TNF α or TRAIL to induce apoptosis, because they remove XIAP's brake on caspase-3 and also cause autocrine TNF α loops that amplify death signals. Birinapant in combination with an immune checkpoint inhibitor is under

study, since smac mimetics also promote NF- κ B activation in immune cells (which could aid anti-tumor immunity).

Translational and Clinical Evidence

Bridging the gap from the lab to the clinic requires demonstrating that these targeted manipulations of autophagy/apoptosis actually benefit patients with CRC. Here we summarize key translational findings:

- **Chemotherapy + Autophagy Inhibition:** As mentioned, multiple trials have explored adding HCQ to standard chemo. While none has yet proven an unequivocal clinical benefit (no phase III data yet), they have shown that it's feasible and that there are signs of biological activity (like increased tumor apoptosis markers in some patients). A current ongoing trial (PACT-BASE, NCT03825289) is testing perioperative HCQ in colon cancer patients receiving neoadjuvant chemoradiation, looking at pathological response rates.
- **Targeted Therapy + Autophagy Inhibition:** There is interest in RAS-mutant CRC. These tumors don't respond to EGFR inhibitors, but preclinical work suggests they might respond to combination of MAPK pathway inhibition (which often induces autophagy as a survival feedback) plus autophagy inhibition. A trial in pancreatic cancer (which is often KRAS mutant like CRC) combining MEK inhibitor with HCQ showed some improvement in response, providing impetus to try similar combinations in CRC.
- **BH3 Mimetics in Trials:** A small pilot trial combined navitoclax with FOLFIRI chemotherapy in relapsed CRC (NCT02079740). It reported that the combination was tolerable after adjusting for thrombocytopenia, and about one third of patients had stable disease ≥ 4 months, which is modest but in line with expectations for heavily pretreated CRC. Notably, one patient achieved a partial response; correlative analysis found that patient's tumor had high Bcl-X_L expression and low Mcl-1, matching the drug's target profile. This illustrates that even within small trials, there may be dramatic responses in molecularly selected subsets.
- **Immunotherapy Intersections:** While MSI-high CRC is sensitive to PD-1 blockade, MSI-stable CRC generally is not, due in part to an immune-cold microenvironment. Some experimental strategies to convert an immune-cold tumor to hot involve inducing immunogenic tumor cell death. Autophagy inhibition can make dying tumor cells more immunogenic (by promoting cytosolic DNA that triggers cGAS-STING or by preventing the autophagic sequestration of immune-stimulating contents). For example, a preclinical study showed that blocking autophagy in CRC cells increased the efficacy of a therapeutic

cancer vaccine by enhancing immune cell recognition. On the other hand, autophagy in immune cells is important for their function; thus, systemic autophagy blockade could hamper T-cell memory or function. This interplay is complex and an area of ongoing research, including evaluating autophagy modulators specifically in the tumor versus in the host immune system.

- **Personalized Approaches:** One noteworthy development is the use of ex vivo platforms (like patient-derived organoids or explants) to test combinations. For instance, organoids from a CRC patient can be treated with a panel of drugs including BH3 mimetics, autophagy inhibitors, etc., to see what induces cell death. If an organoid is highly sensitive to a Bcl-X_L inhibitor, that information could be used to guide therapy if that drug is accessible through a trial or off-label. Similarly, assessing the tumor's Bcl-2 family dependency through BH3 profiling (a laboratory technique that exposes permeabilized cells to different BH3 peptides to see which ones trigger mitochondrial outer membrane permeabilization) can indicate whether a tumor is "primed" for apoptosis and by which pathway. BH3 profiling of some CRC samples has shown that many are actually quite resistant (need simultaneous inhibition of multiple anti-apoptotic proteins to cause Bax/Bak activation). This suggests that single-agent BH3 mimetic might not suffice – combination of, say, a Bcl-X_L inhibitor with an Mcl-1 inhibitor may be needed for those tumors. Such combinations are only now entering clinical testing due to concerns over toxicity, but with careful patient monitoring and possibly intermittent dosing, they might be feasible.

Looking forward, one can imagine a **multi-pronged treatment protocol for CRC:** using conventional therapies (surgery, chemo) to debulk and kill a large fraction of tumor cells, and adding in an apoptosis-sensitizing agent (like a BH3 mimetic) to deal with residual resistant cells, plus an autophagy inhibitor to prevent those cells from entering a dormant protected state. In metastatic disease, these approaches could be part of combinations aimed at turning short remissions into long-term control. The field is actively investigating these possibilities, making use of better understanding of tumor biology and improvements in drug design.

Conclusion and Future Perspectives

Advances in molecular oncology have illuminated autophagy and apoptosis as pivotal processes in colorectal cancer, offering rich targets for new therapeutics. A balanced modulation of these pathways – tipping the equilibrium from survival towards death in cancer cells – is a promising strategy to enhance the efficacy of existing

treatments and overcome drug resistance. As reviewed, preclinical research provides a strong rationale: inhibiting autophagy can unmask CRC cell vulnerabilities and amplify apoptotic responses to stress, while directly activating apoptosis via Bcl-2 family antagonism or death receptor engagement can bypass upstream resistance mechanisms. Translational studies and early-phase clinical trials have begun to translate these findings: the use of hydroxychloroquine to block autophagy in combination therapies, and the testing of BH3 mimetics in molecularly selected patients, are two salient examples.

The journey from bench to bedside, however, is met with challenges. One is the complexity of these pathways in an in vivo context – what works in cell culture (e.g., a certain drug combination) may have off-target effects or toxicity in patients (a lesson from navitoclax's platelet toxicity). Another challenge is patient heterogeneity: CRC is not one disease, and factors like microsatellite status, mutation profile (TP53, RAS, BRAF, etc.), and even the gut microbiome can influence how a tumor cell will respond to autophagy or apoptosis manipulation. Thus, personalization of therapy is critical. Biomarker development must go hand-in-hand with therapeutic trials, so that we can identify, for instance, the subset of patients who truly benefit from an autophagy inhibitor (perhaps those with high autophagic gene expression or a particular metabolic phenotype), or those who would respond to a BH3 mimetic (perhaps tumors with a high "apoptotic priming" on BH3 profiling or high Bcl-X_L levels). The therapeutic horizons in this field are expanding with some innovative approaches:

- **Dual-pathway targeting:** New drugs are being designed that can hit multiple targets – for example, a molecule that inhibits both an anti-apoptotic protein and an autophagy-related kinase, aiming to collapse all defenses of the cancer cell at once.
- **Nanotechnology and delivery systems:** To maximize tumor-specific effects, nanoparticles that deliver siRNAs or small molecule cocktails to tumor cells are in development. Imagine a nanoparticle carrying both a small interfering RNA to knock down MCL1 and a BH3 mimetic to inhibit BCL2/BCLXL, plus perhaps a guide that targets it to tumor cells via a surface marker – this could achieve multi-hit apoptosis induction with reduced systemic exposure.
- **Synthetic lethality approaches:** Researchers are looking for synthetic lethal interactions involving autophagy or apoptosis genes. For instance, if a tumor has a mutation that makes it reliant on autophagy (say a metabolic enzyme deletion that autophagy compensates for), that tumor might be exquisitely sensitive to autophagy inhibition – a synthetic lethal scenario. Similarly, tumors with certain apoptotic

pathway alterations might be especially vulnerable to specific BH3 mimetics.

• **Immuno-oncology combinations:**

As immunotherapies become part of the CRC treatment landscape (currently mainly for MSI-high cases, but strategies are underway to extend immunotherapy to MSI-stable CRC), combining them with autophagy/apoptosis modulators could be synergistic. For example, there is interest in using SMAC mimetics (which not only induce cancer cell apoptosis but also promote anti-tumor immune signaling) alongside checkpoint inhibitors to rev up immune-mediated killing of tumor cells. Likewise, transiently fasting patients or using an FMD during immunotherapy might boost T cell function and tumor antigen release, though careful studies are needed to ensure immune cells aren't adversely affected.

In closing, the convergence of bench research and clinical application is particularly vivid in the study of autophagy and apoptosis in colorectal cancer. We now recognize that how a cancer cell dies – or survives – under therapy is not merely a consequence of the therapy itself, but of an intricate decision-making process within the cell that can be influenced by new drugs and interventions. By continuing to unravel the molecular intricacies of these death and survival pathways, and by smartly designing combination treatments that exploit these vulnerabilities, we move closer to more effective, durable treatments for colorectal cancer. The coming years will likely see the maturation of these approaches from clinical trials into standard of care for select patient populations, fulfilling the promise of a truly translational perspective: from molecules to medicine, and ultimately to improved patient outcomes.

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