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Intermittent Fasting and The Multifaceted Role of Beclin-1 and Bcl-2 In Regulating Autophagy and Apoptosis in Colorectal Cancer

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ABSTRACT

Intermittent fasting (IF) has emerged as a promising adjunct strategy in cancer therapy due to its profound effects on cellular metabolism and stress responses. This review examines how IF influences the balance between autophagy and apoptosis in colorectal cancer (CRC), with a particular focus on the dual roles of Beclin-1 and Bcl-2. Beclin-1 is a core autophagy regulator that can act as a tumor suppressor, while Bcl-2 is an anti-apoptotic oncoprotein that also inhibits autophagy by binding Beclin-1. We discuss the molecular interplay between Beclin-1 and Bcl-2 and how IF modulates these pathways, drawing on evidence from preclinical models and emerging clinical observations. IF triggers nutrient-sensing pathways (e.g., lowering insulin/IGF-1, mTOR signaling) that induce autophagy and can sensitize cancer cells to apoptotic stimuli. Under fasting conditions, modifications such as JNK1-mediated phosphorylation of Bcl-2 disrupt the Bcl-2–Beclin-1 complex, thereby unleashing autophagy. The consequences are multifaceted: autophagy activation can promote cancer cell survival under metabolic stress, but prolonged or excessive autophagy may lead to type II cell death and enhance apoptotic signaling. In CRC, Beclin-1 expression is frequently dysregulated, and Bcl-2 family proteins are often upregulated to evade apoptosis. IF may counteract these pro-tumorigenic adaptations by simultaneously inducing autophagic self-digestion of cellular components and lowering the threshold for apoptosis in cancer cells. We highlight recent studies showing that short-term fasting or fasting-mimicking diets synergize with chemotherapy in CRC models by enhancing apoptosis and inhibiting tumor growth. The therapeutic implications of targeting the Beclin-1/Bcl-2 axis are also discussed, including the potential of combining IF with agents that modulate autophagy or Bcl-2 family function. A balanced evaluation of preclinical evidence and early clinical data suggests that IF, through its modulation of Beclin-1 and Bcl-2 pathways, holds promise for improving colorectal cancer treatment outcomes, although careful patient selection and protocol design are needed to maximize benefits and minimize risks.

KEY WORDS: Beclin-1 and Bcl-2 pathways, colorectal cancer, Intermittent fasting.

INTRODUCTION

Colorectal cancer remains a major health challenge worldwide, prompting exploration of novel strategies to enhance treatment efficacy and patient outcomes. Autophagy and apoptosis – the two fundamental cell fate pathways – are central to cancer biology and therapy response. Autophagy is a lysosomal degradation process that can either suppress tumor initiation by removing damaged organelles and DNA, or promote tumor survival by helping cancer cells endure stress. Apoptosis is a programmed cell death mechanism that eliminates damaged or unwanted cells, and its evasion is a hallmark of cancer. The Bcl-2 protein family plays a pivotal role in apoptosis regulation, with anti-apoptotic members (like Bcl-2 itself) preventing cell death by sequestering pro-apoptotic factors. Intriguingly, Bcl-2 also intersects with the autophagy machinery: it binds to Beclin-1 (the mammalian autophagy protein essential for autophagosome formation) and inhibits autophagy. This dual role positions Bcl-2 as a molecular switch between survival pathways, while Beclin-1 stands at the crossroads of autophagy and cell death decisions. In colorectal cancer,

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alterations in both autophagy and apoptosis pathways contribute to tumorigenesis and therapy resistance. For instance, Beclin-1 has been regarded as a haploinsufficient tumor suppressor in several cancers, and its expression or activity may be perturbed in CRC; meanwhile, overexpression of Bcl-2 or related anti-apoptotic proteins can render colorectal tumors resistant to chemotherapy-induced apoptosis. Understanding how these pathways can be modulated is critical for developing new therapeutic approaches.

Intermittent fasting – periodic cycles of severe caloric restriction or no caloric intake – influences many of the same cellular pathways that regulate autophagy and apoptosis. Nutrient deprivation is a natural trigger for autophagy, as cells attempt to recycle internal resources, and can also modulate apoptotic thresholds. IF has attracted attention in oncology for its potential to sensitize cancer cells to treatment while protecting normal cells, a concept termed "differential stress resistance". In this review, we examine mechanisms by which intermittent fasting impacts autophagy and apoptosis in colorectal cancer, highlighting the multifaceted role of Beclin-1 and Bcl-2. We integrate evidence from molecular studies, animal models of fasting and cancer, and clinical or translational studies of fasting in cancer patients. Key questions include: How does IF-induced metabolic stress activate autophagy in CRC cells? In what ways do Beclin-1 and Bcl-2 mediate the crosstalk between autophagy and apoptosis under fasting conditions? And can leveraging this crosstalk translate into therapeutic gains in CRC? By addressing these points, we aim to provide a comprehensive picture of the Beclin-1/Bcl-2 axis under intermittent fasting and to outline emerging therapeutic implications.

Table 1: Key Roles of Beclin-1 and Bcl-2 in Autophagy and Apoptosis

Protein	Function in Autophagy	Function in Apoptosis	Regulation by IF
Beclin-1	Initiates autophagosome formation	Indirect (via autophagy regulation)	Released from Bcl-2 under stress
Bcl-2	Binds Beclin-1 and inhibits autophagy	Inhibits pro-apoptotic proteins (Bax/Bak)	Phosphorylation by JNK1 reduces binding
Bcl-X_L	Inhibits autophagy via Beclin-1 binding	Anti-apoptotic, compensates Bcl-2 loss	Less responsive to JNK1; affected by DAPK
Mcl-1	Inhibits autophagy (indirect)	Strongly anti-apoptotic, especially in CRC	Rapidly downregulated during fasting

Table 2: Effects of Intermittent Fasting on CRC Cell Fate Pathways

Parameter	Normal Cells	CRC Cells
Growth Factor Availability	Reduced	Reduced
mTOR Activity	Suppressed	Suppressed
Autophagy	Adaptive	Initially protective, later lethal
Apoptosis Sensitivity	Decreased	Increased
Cellular Outcome	Protection from chemo-toxicity	Enhanced response to therapy

Intermittent Fasting, Metabolic Stress, and Autophagy in Cancer

IF as a Modulator of Autophagy: Intermittent fasting imposes recurrent episodes of nutrient and growth factor deprivation. At the cellular level, fasting drives a metabolic switch from an anabolic state (promoted by constant food intake) to a catabolic, stress-resistant state. In normal cells, scarcity of glucose and growth factors (like insulin and IGF-1) leads to reduced activation of pro-growth pathways such as PI3K/Akt/mTOR, and increased activation of stress-responsive kinases such as AMP-activated protein kinase (AMPK). The net effect is the induction of autophagy as an adaptive mechanism to maintain energy homeostasis by recycling intracellular components. Indeed, **short-term fasting is a potent trigger of autophagy** in various tissues. Classic studies showed that even 24–48 hours of fasting can markedly increase autophagic vesicle formation in cells. For example, a 48-h fast in mice induces "profound" autophagy in neurons, and fasting similarly activates autophagy in liver and other organs as cells respond

to nutrient stress. This autophagy induction under fasting is largely mediated by inhibition of mTOR (a negative regulator of autophagy) and activation of autophagy-initiating complexes that involve Beclin-1. Beclin-1 is a key scaffold protein for the class III PI3-kinase (Vps34) complex required to form autophagosomes, and thus is central to autophagy initiation. Under nutrient-rich conditions, Beclin-1 activity is restrained by binding to Bcl-2 and by growth signals that favor cell proliferation over catabolism. IF can tilt this balance: by lowering insulin/IGF-1 and related signals, IF diminishes the PI3K/Akt pathway activity and relieves mTOR-mediated autophagy suppression. Furthermore, energetic stress from fasting activates JNK1 and other kinases that post-translationally modify autophagy regulators (discussed below). Consequently, intermittent fasting provides pulses of autophagy activation in cells, which may have significant implications for cancer outcomes.

Dual Role of Autophagy in Tumor Biology: It is important to recognize that autophagy in cancer is context-dependent, often described as a "double-edged sword." In early stages of tumorigenesis, autophagy has a tumor-suppressive function by removing damaged organelles, protein aggregates, and unstable genomic elements that could otherwise contribute to malignant transformation. Beclin-1 itself was first identified as a tumor suppressor when monoallelic deletion of its gene (BECN1) was noted in breast and ovarian cancers. Consistently, loss of autophagy genes can lead to accumulation of oxidative damage and genomic instability in normal cells, promoting cancer development. In CRC, however, the role of autophagy appears more complex. Some studies indicate *reduced* expression or function of autophagy components (including Beclin-1, UVRAG, ATG5, etc.) can facilitate tumor initiation. On the other hand, once a tumor is established, cancer cells can hijack autophagy as a survival mechanism under stress conditions such as hypoxia, starvation, or chemotherapy exposure. In line with this, high autophagy activity has been correlated with aggressive disease or therapy resistance in CRC. For example, LC3-II (an autophagosome marker) is often elevated in advanced colorectal tumors, and high LC3 correlates with poorer prognosis. CRC cells with constitutive autophagy may better withstand nutrient deprivation and resist apoptosis induced by treatments. Thus, intermittent fasting's induction of autophagy could have complex effects: it might suppress tumor growth by enforcing a non-permissive metabolic state, yet it might also transiently help cancer cells survive the fasting period. The net impact likely depends on fasting duration, timing relative to therapy, and tumor-specific factors (genotype, stage, microenvironment). Preclinical evidence suggests that fasting generally **sensitizes** tumors to therapy, implying that any pro-survival autophagy triggered in cancer cells is ultimately overwhelmed by enhanced apoptosis or impaired recovery when nutrients return.

IF and Differential Stress Responses: A remarkable aspect of short-term fasting is its differential effect on normal vs. cancer cells. Normal cells enter a maintenance mode during fasting – reducing protein synthesis, dividing less, and enhancing DNA repair – which makes them more resistant to stresses like chemotherapy. Cancer cells, due to oncogenic signals, fail to adequately slow their metabolism and continue trying to proliferate even in low-nutrient conditions. This mismatch creates a vulnerability in cancer cells: fasting can exacerbate metabolic stress in tumors and push them towards cell death, particularly when combined with cytotoxic drugs. Raffaghello et al. demonstrated that 48 hours of fasting protected mice's normal cells but not cancer cells from high-dose chemotherapy, resulting in reduced treatment toxicity and maintained anti-tumor efficacy. IF's protective effect on normal tissue (and lack thereof in tumor) has been attributed to lowered growth factors and activation of stress resistance pathways in healthy cells. At the same time, fasting tumor cells experience an "energy crisis" – for instance, a fasting-induced anti-Warburg effect has been observed, wherein glucose uptake and glycolysis in the tumor are acutely reduced, forcing a reliance on mitochondrial respiration and increasing reactive oxygen species production. In a mouse CRC model (CT26 colon carcinoma), 2-day fasting combined with chemotherapy (oxaliplatin) significantly enhanced tumor growth suppression compared to chemotherapy alone. Mechanistically, fasting reduced glucose availability and downregulated GLUT1/GLUT2 transporters on cancer cells, leading to lower glycolytic flux. This metabolic switch increased mitochondrial oxidant production and DNA damage in cancer cells, thereby promoting apoptosis in the tumor. These findings underscore how IF can pressure cancer metabolism and tilt the balance toward cell death rather than survival. Notably, autophagy is one means by which cancer cells attempt to cope with such stress (by breaking down intracellular stores to fuel metabolism and mitigate damage). However, when stress is intense or prolonged, autophagy may not suffice or may even contribute to cell death (sometimes described as autophagic cell death). Excessive or sustained autophagy can lead to self-consumption of vital cellular components, a state that can cooperate with or transition into apoptosis. Indeed, there is evidence that prolonged fasting or calorie restriction could induce

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a form of type II autophagic cell death in cancer cells under certain conditions. Thus, IF may set into motion a two-phase effect: an early autophagy activation as an adaptive response, followed by apoptosis if the stress is unrelieved or if pro-death signals accumulate. The presence of functional apoptotic pathways (e.g., intact p53, Bax/Bak function) in the cancer cell will influence this outcome.

Beclin-1 and Bcl-2: Molecular Interactions at the Crossroads of Autophagy and Apoptosis

Beclin-1: Autophagy Initiator and Contextual Tumor Suppressor: Beclin-1 (BECN1 gene) is the central component of the class III PI3K autophagy initiation complex. Through its coiled-coil and domain interactions, Beclin-1 recruits Vps34 and other autophagy proteins to form the pre-autophagosomal structure. Uniquely, Beclin-1 contains a BH3-like motif, making it structurally similar to pro-apoptotic BH3-only proteins. However, full-length Beclin-1 does not directly induce apoptosis; rather, this BH3 domain's main function is to bind to Bcl-2 family proteins. Through this interaction, Beclin-1 becomes a node of crosstalk: when Beclin-1 is bound by anti-apoptotic proteins like Bcl-2 or Bcl-X_L, its autophagy function is inhibited. In essence, Bcl-2 sequesters Beclin-1 away from the autophagy initiation complex, thereby suppressing autophagy. This mechanism was first elucidated by Pattingre *et al.* in 2005, who showed that Bcl-2 directly binds Beclin-1 and that Bcl-2 overexpression can block autophagy induction. Normally, cells can release Beclin-1 from Bcl-2 under stress via several mechanisms: one key mechanism is phosphorylation of Bcl-2 by stress kinases, which reduces Bcl-2's binding affinity for Beclin-1. Another is competition from other BH3-only proteins which, during cellular stress, can occupy Bcl-2's binding groove and displace Beclin-1.

Bcl-2: Guardian of Survival and Autophagy Brake: Bcl-2 is best known for its role in guarding against apoptosis. Located on the outer mitochondrial membrane, Bcl-2 (along with its homologs like Bcl-X_L and Mcl-1) binds and neutralizes pro-apoptotic proteins (Bax, Bak, and BH3-only activators) to prevent mitochondrial outer membrane permeabilization and cytochrome c release. This anti-apoptotic function is a major reason Bcl-2 is considered an oncogene – many cancers, especially hematologic malignancies, overexpress Bcl-2 to evade cell death. In solid tumors like CRC, Bcl-2 expression is more variable (we discuss CRC-specific patterns below), but the principle remains that high Bcl-2 activity tilts the cell toward survival. Concurrently, Bcl-2's binding to Beclin-1 means that high Bcl-2 also serves as an "autophagy brake." Under non-stressed conditions, a fraction of Bcl-2 at the endoplasmic reticulum forms a complex with Beclin-1, keeping autophagy at basal levels. When cells are starved or otherwise stressed, signaling pathways converge to relieve this inhibition. For example, c-Jun N-terminal kinase 1 (JNK1) is activated by starvation and phosphorylates Bcl-2 on three residues (T69, S70, S87) in its unstructured loop region. Phosphorylated Bcl-2 can no longer bind tightly to Beclin-1, causing the Beclin-1–Bcl-2 complex to dissociate. This event unleashes Beclin-1 to trigger autophagy. Notably, mutant Bcl-2 that cannot be phosphorylated (Bcl-2-AAA) fails to release Beclin-1 during starvation and continues to block autophagy. Conversely, a phosphomimetic Bcl-2 mutant (Bcl-2-EEE) that mimics constitutive JNK1 phosphorylation cannot bind Beclin-1 even in full-nutrient conditions, and thus it cannot inhibit autophagy. These elegant experiments confirm that post-translational modification of Bcl-2 is a critical switch enabling autophagy in response to fasting or other stresses. Other modifications and interactions modulate the Bcl-2–Beclin-1 axis as well. For instance, death-associated protein kinase (DAPK) can phosphorylate Beclin-1 on Thr119 (within the BH3 domain of Beclin) to weaken its interaction with Bcl-2/Bcl-X_L. Thus, both "sides" of the complex (Bcl-2 and Beclin-1) have regulatory inputs that determine whether they remain bound or separate during cellular stress.

Autophagy vs Apoptosis: The Balance of Binding: The interplay between autophagy and apoptosis pathways often hinges on common regulators. Bcl-2 family proteins are prime examples, as they can simultaneously control both processes by their choice of binding partners. When Bcl-2 is occupied sequestering Beclin-1, it might be less available to bind pro-apoptotic Bax/Bak or BH3-only proteins – but usually Bcl-2 is abundant enough to engage in multiple interactions. Intriguingly, studies have shown a temporal difference in how starvation releases Beclin-1 vs. pro-apoptotic proteins from Bcl-2. During nutrient deprivation, Beclin-1 is freed from Bcl-2 relatively early (within a few hours), allowing autophagy to commence, whereas Bcl-2's grip on Bax is maintained until stress is more prolonged. In one study, 4 hours of starvation induced Beclin-1–Bcl-2 dissociation (autophagy on), but it took ~16

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hours of starvation for the Bcl-2–Bax association to significantly disrupt and for apoptosis to be triggered, correlating with caspase-3 activation at those later time points. This staged response suggests cells preferentially activate autophagy as a first-line survival strategy under stress; only if the stress continues or intensifies do they tip into apoptosis. Bcl-2 may act as a buffer during short-term stress – giving autophagy a chance to mitigate damage – but with prolonged stress, modifications (like persistent JNK activation) eventually also neutralize Bcl-2's anti-apoptotic function, permitting cell death. This model aligns well with what might happen during fasting in a cancer: *transient fasting (short enough to be manageable by the tumor) may primarily induce autophagy, but more prolonged or repeated fasting, especially with therapeutic interventions, could overcome Bcl-2's protections and induce apoptosis in cancer cells.* From a treatment perspective, it might be advantageous to combine fasting with agents that either boost this dissociation (promote autophagy and subsequent apoptosis) or directly inhibit Bcl-2, as discussed later.

Beclin-1 and Bcl-2 in Colorectal Cancer: Alterations in the Beclin-1/Bcl-2 axis have been documented in CRC patient tumors. Beclin-1 expression in CRC is often dysregulated: immunohistochemical studies show that about 15–20% of colorectal cancers have loss of Beclin-1 expression, while another ~20% show marked overexpression of Beclin-1 relative to normal tissue. Intriguingly, both extremes (too little or too much Beclin-1) were associated with worse patient outcomes. In a cohort of 155 CRC patients, those whose tumors either underexpressed Beclin-1 or had very high Beclin-1 had significantly poorer overall survival compared to those with intermediate (normal-like) Beclin-1 levels. The poor-prognosis *low-Beclin-1* group likely reflects a loss of autophagy's tumor suppressive function, which may allow accumulation of genomic damage and also *promote anti-apoptotic pathways in the tumor*. Indeed, loss of Beclin-1 could tilt the balance toward unchecked Bcl-2 activity, enhancing cell survival. On the other hand, the poor-prognosis *high-Beclin-1* group was associated with hypoxic, acidic tumors and high HIF1α levels, suggesting that these tumors are under significant metabolic stress and may be relying on autophagy for survival. High Beclin-1 in an aggressive tumor might indicate that autophagy is helping cancer cells resist cell death (for example, by removing damaged mitochondria or proteins that would otherwise trigger apoptosis). These clinical correlations underscore that Beclin-1 has a context-dependent role in colorectal cancer: it can be tumor-suppressive, but in certain advanced cancer contexts, autophagy (via high Beclin-1 activity) can contribute to tumor progression and treatment resistance.

Bcl-2 family proteins are also dysregulated in CRC, but interestingly, the prototypical Bcl-2 protein itself is not uniformly overexpressed in colorectal cancer. In fact, multiple studies have found that higher Bcl-2 expression in CRC tumors correlates with *better* patient prognosis. A meta-analysis concluded that Bcl-2 positivity is a favorable prognostic factor in CRC, contrary to what is seen in many other malignancies. This seemingly paradoxical finding is explained by the observation that Bcl-2 is often expressed in early-stage or well-differentiated colorectal tumors (and in normal colonic epithelium), but as tumors progress to late stages, they tend to lose Bcl-2 expression in favor of other anti-apoptotic mechanisms. In advanced CRC and especially in chemotherapy-resistant disease, other Bcl-2 family members like Bcl-X_L and Mcl-1 become more critical. For example, colorectal cancer cell lines that acquire resistance to 5-fluorouracil (5-FU) have been shown to dramatically upregulate Bcl-X_L while actually downregulating Mcl-1, suggesting Bcl-X_L is a key survival factor in that context. Patient studies similarly indicate that a subset of CRC overexpress Bcl-X_L, which is associated with resistance to apoptosis and poor response to therapy. Mcl-1, another anti-apoptotic protein, is frequently elevated in various cancers and is noted to contribute to colorectal cancer cell survival, particularly in tumors with certain oncogenic drivers (e.g., BRAF^V600E upregulates Mcl-1 as an escape mechanism from targeted therapy). The interplay between Beclin-1 and these Bcl-2 family proteins in CRC is less charted, but one can speculate: if a CRC predominantly relies on Bcl-X_L or Mcl-1 for survival, the Beclin-1 interaction may shift towards those proteins instead of Bcl-2. (Bcl-X_L and Mcl-1 also bind Beclin-1's BH3 domain and can inhibit autophagy.) Notably, Bcl-X_L lacks the same phosphorylation sites as Bcl-2 and thus may not respond to stress in the same way; alternative mechanisms (like DAPK-mediated Beclin-1 phosphorylation mentioned above) can disrupt Beclin-1 binding to Bcl-X_L. The net effect in CRC cells is that high levels of anti-apoptotic Bcl-2 family proteins (whether Bcl-2, Bcl-X_L, or Mcl-1) tend to correlate with suppressed autophagy and apoptosis, fostering resistance. In line with this, experiments show that downregulating Bcl-2 or Bcl-X_L can trigger autophagy and/or apoptosis in CRC cells, leading to cell death. For instance, silencing Bcl-X_L via siRNA in 5-FU-resistant CRC cells substantially inhibited their growth and made them more prone to apoptosis.

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Similarly, knockdown or chemical inhibition of Bcl-2 in CRC cell models has been reported to induce autophagy and cell death, particularly when used in combination with chemotherapeutic drugs.

Intermittent Fasting Influences on Beclin-1/Bcl-2 and Cell Fate in CRC

Fasting Releases the Autophagy Brake: Given the molecular details discussed, it becomes evident how intermittent fasting might impact the Beclin-1/Bcl-2 axis in colorectal cancer cells. By rapidly lowering available glucose and growth factors, fasting will activate stress kinases (like JNK1, AMPK) in cancer cells. JNK1 activation in fasting conditions can lead to Bcl-2 phosphorylation in CRC cells just as in other cell types. We anticipate that in a CRC cell undergoing IF, JNK-mediated Bcl-2 phosphorylation and perhaps DAPK-mediated Beclin-1 phosphorylation combine to disrupt Beclin-1's association with Bcl-2/Bcl-X_L. Indeed, a recent study in mice showed that 48-hour fasting-induced autophagy is blunted if Bcl-2 is overexpressed, confirming that Bcl-2 is a limiting factor for autophagy during fasting. Conversely, when Beclin-1 is mutated so that it cannot bind Bcl-2, mice exhibit increased autophagy and are better able to withstand metabolic challenges. These findings strongly suggest that fasting promotes autophagy in part by functionally inactivating the Bcl-2 brake on Beclin-1. In the context of a CRC tumor, if fasting can induce even a transient surge of autophagy, this may have several consequences: (1) It could degrade intracellular assets of the cancer cell (e.g., consume stored nutrients, degrade pro-survival proteins or even some oncogenic proteins), weakening the cell; (2) It may initially protect the cell from apoptosis (by clearing damaged components), but if fasting is prolonged or combined with therapy, the autophagy may shift to a death modality; (3) It can potentially expose vulnerabilities – for example, a cell heavily reliant on autophagy for survival may become more sensitive to autophagy inhibitors or to subsequent nutrient restoration (maladaptive refeeding injury).

Sensitization to Apoptosis: A major rationale for using IF in cancer therapy is to sensitize cancer cells to apoptosis induced by chemotherapy or other treatments. IF influences many apoptotic regulators: it tends to decrease expression of pro-survival factors (e.g., Mcl-1, which has a short half-life and is lost rapidly in the absence of protein synthesis) and can increase pro-apoptotic signals (e.g., elevated oxidative stress can activate p53 or BH3-only proteins). One study noted that fasting upregulated the tumor suppressor protein p53 and pro-apoptotic mediators in cancer cells while downregulating Mcl-1, thereby priming cells for apoptosis when exposed to chemo. Additionally, fasting or fasting-mimicking diets have been shown to reduce levels of circulating growth factors like IGF-1 by 50–70%, which in turn diminishes Akt signaling in tumors and can lead to lower Bcl-2/Bcl-X_L expression (since the PI3K/Akt pathway often drives transcription of survival genes). In support of this, Zhao *et al.* found that serum from fasted animals, when added to cancer cells, induced more apoptosis than serum from fed animals, suggesting that systemic factors during fasting are less supportive of tumor cell survival. Importantly, fasting *in vivo* in CRC models has demonstrated pro-apoptotic effects: for instance, Cheng *et al.* observed increased cleaved caspase-3 in colorectal tumors of fasted mice, indicating higher apoptotic activity (especially when fasting was combined with chemotherapy). In our aforementioned CT26 colon cancer example, fasting potentiated oxaliplatin-induced apoptosis, as evidenced by greater tumor cell death and regression.

What role do Beclin-1 and Bcl-2 specifically play in this fasting-induced apoptosis? While autophagy activation via Beclin-1 might at first delay apoptosis (by mitigating cellular stress), the concurrent inhibition of Bcl-2's anti-apoptotic function (through JNK1 phosphorylation or downregulation of Bcl-2 expression) will tip the scales toward apoptosis. Essentially, fasting can *simultaneously* promote autophagy **and** reduce the apoptotic threshold by disabling Bcl-2. There is also interplay in the sense that autophagy may consume certain apoptosis inhibitors. For example, autophagy can degrade proteins like p62 or damaged mitochondria; loss of mitochondria might directly lower Bcl-2 levels (since some Bcl-2 is mitochondrial) and release pro-apoptotic factors. Moreover, a phenomenon called autophagy-associated cell death has been observed wherein extensive autophagy leads to self-digestion and activation of caspase-independent death. In practice, however, most often autophagy and apoptosis work in concert under severe stress: autophagy may initiate as a survival attempt and then apoptosis executes cell death if the stress cannot be resolved.

Preclinical and Clinical Evidence in CRC: Multiple preclinical studies have tested IF or calorie restriction in colorectal cancer models. In addition to the CT26 murine model, fasting has been shown to inhibit the growth of human CRC xenografts. In one study, a fasting-mimicking diet caused a significant reduction in tumor progression and enhanced the efficacy of 5-FU in a xenograft model, correlating with increased markers of apoptosis in tumor tissues (TUNEL staining) and decreased Ki-67 proliferation index. Another study found that alternate-day fasting in APC^Min mice (a model of intestinal tumorigenesis) led to fewer and smaller tumors, suggesting autophagy induction and possibly elimination of tumor-initiating cells. On the cellular level, CRC cell lines subjected to serum/glucose deprivation (to mimic fasting) show increased autophagosome formation (LC3 puncta) and also an increase in pro-apoptotic factors like Bim and cleaved PARP over time. Notably, combining nutrient deprivation with chemotherapy yields a greater apoptotic fraction than chemotherapy alone. This is consistent with reports that fasting makes colorectal cancer cells more susceptible to chemotherapeutic apoptotic stimuli.

From the perspective of Beclin-1 and Bcl-2, some studies have measured these proteins under fasting conditions. For example, Liang *et al.* reported that nutrient deprivation in colon cancer cells led to a time-dependent increase in Beclin-1 levels and a decrease in the Bcl-2/Beclin-1 complex formation, as detected by co-immunoprecipitation. In parallel, pro-apoptotic changes such as Bax activation were seen after longer deprivation. In an interesting rat model of intermittent fasting combined with a carcinogen (to induce colon cancer), investigators found that IF upregulated the expression of autophagy genes (Beclin-1, LC3) in colonic mucosa while also reducing Bcl-2 levels, resulting in higher rates of cancer cell apoptosis. These mechanistic findings align well with the conceptual framework that IF pushes cancer cells to a state of autophagy-driven catabolism and primes the intrinsic apoptotic pathway by modulating Bcl-2 family function.

On the clinical side, data are still nascent but evolving. Small clinical studies and case series have explored fasting in cancer patients, including those with colorectal cancer. In a pilot case series involving various solid tumor patients (including CRC) who voluntarily fasted 1–2 days before and after chemotherapy, patients reported fewer side effects and no evidence of compromised treatment efficacy. Though primarily focused on tolerability, this study hinted that short-term fasting is safe in the oncology setting. Ongoing clinical trials specifically in CRC are examining fasting or fasting-mimicking diets in combination with chemotherapy (e.g., a trial of 36-hour water-only fasting around each chemotherapy cycle for stage II/III CRC patients receiving CAPOX chemotherapy). While results are pending, the hypothesis is that fasting will not only protect normal cells (reducing chemo toxicity) but also **sensitize tumor cells to apoptosis, thereby improving treatment response**. Correlative endpoints in such trials include measuring levels of apoptosis markers and autophagy markers in patient tumor samples or blood. One recent window-of-opportunity trial in patients with colorectal liver metastases tested a 3-day fasting-mimicking diet prior to surgery; analysis of resected tumors showed increased expression of genes related to autophagy and cell death, suggesting the fasting intervention did impact tumor biology as expected.

In summary, intermittent fasting imposes a profound metabolic challenge to colorectal cancer cells, which appears to activate autophagy through Beclin-1 while at the same time undermining Bcl-2's anti-apoptotic hold on the cells. The result is a time-dependent shift from cell survival toward cell death. In the next section, we consider how these insights might be translated into therapeutic strategies, including how manipulating the Beclin-1/Bcl-2 axis could enhance colorectal cancer treatments.

Therapeutic Implications and Future Directions

The intersection of intermittent fasting with the Beclin-1/Bcl-2 regulatory axis offers several intriguing therapeutic angles for colorectal cancer:

- **Fasting as an Adjunct to Chemotherapy:** Perhaps the most immediate application is incorporating short-term IF (or fasting-mimicking diets) alongside standard chemotherapy to improve outcomes. As discussed, fasting can sensitize cancer cells to drugs like 5-FU and oxaliplatin by lowering the apoptotic threshold. A practical example is combining a 24–48 hour fast with the administration of chemotherapy, timed such that

the patient is in a fasted state during drug infusion and for some hours after. Preclinical data strongly support that this approach increases tumor cell kill via enhanced apoptosis. Clinically, early-phase trials (e.g., NCT01802346) are testing tolerability and preliminary efficacy of fasting with chemo in CRC patients. The expectation is that fasting will transiently suppress pro-survival signals (IGF-1/Akt/Bcl-2) and induce autophagy, making the cancer cells more prone to DNA damage and apoptotic triggers from chemotherapy, while normal cells are in a protected quiescent mode. If successful, this could translate into higher tumor response rates or allowing lower chemotherapy doses to be used (for similar effect but with less toxicity). However, challenges include ensuring patient adherence to fasting and managing potential side effects like fatigue or weight loss from repetitive fasting – especially in cancer patients who may be prone to cachexia. Future studies are needed to optimize fasting duration and frequency (e.g., fasting only before every other chemo cycle vs. every cycle) and to identify which patients benefit most (perhaps those with certain metabolic profiles or tumors highly dependent on the pathways fasting targets).

- **Targeting the Bcl-2/Beclin-1 Interaction:** The knowledge that Bcl-2 inhibits autophagy by binding Beclin-1 suggests a druggable node. In fact, small-molecule *BH3 mimetics*, developed to inhibit Bcl-2's anti-apoptotic function, can also disrupt Bcl-2's interaction with Beclin-1. For instance, the compound ABT-737 and its clinical analog navitoclax (ABT-263) bind with high affinity to Bcl-2 and Bcl-X_L, displacing endogenous BH3 domain proteins (including Beclin-1's BH3 domain). While the primary outcome of BH3 mimetics is to induce apoptosis (by freeing pro-apoptotic Bax/Bak from Bcl-2/Bcl-X_L), a secondary effect is often the induction of autophagy due to Beclin-1 release. This was actually observed in some leukemia models where ABT-737 treatment triggered a protective autophagy response in cancer cells, potentially offsetting some of its lethal effect. In colorectal cancer, BH3 mimetic drugs are still experimental but hold promise. A **2020 study by Ramesh and Medema** highlighted that many CRC tumors have an imbalance in Bcl-2 family proteins that could be exploited with BH3 mimetics. For example, tumors with high Bcl-X_L are candidates for Bcl-X_L-specific inhibitors. Indeed, a novel BH3 mimetic, A-1331852, that selectively targets Bcl-X_L was shown to strongly suppress growth of 5-FU–resistant CRC cells by inducing apoptosis. Similarly, inhibitors targeting Mcl-1 (such as S63845) are being tested preclinically to counteract Mcl-1–mediated chemo-resistance in CRC. The interplay with autophagy here is double-edged: using BH3 mimetics will unleash Beclin-1. In some contexts, that might be beneficial (pushing cells into autophagy-related death), but it could also allow tumor cells to survive longer via autophagy. Therefore, a compelling strategy is **combining BH3 mimetics with autophagy inhibitors**. In preclinical experiments, adding chloroquine (an autophagy inhibitor) to navitoclax led to greater cell death in CRC cell cultures, suggesting synergy when both apoptosis and autophagy are co-targeted. From a translational perspective, BH3 mimetics such as **venetoclax** (Bcl-2 inhibitor) have revolutionized treatment of leukemias, and there is interest in repurposing them for solid tumors. Some early-phase trials are exploring venetoclax in gastrointestinal malignancies in combination with conventional chemotherapy or targeted agents. Based on the CRC-specific biology, it may be more effective to use a Bcl-X_L inhibitor or a dual Bcl-2/Bcl-X_L inhibitor (navitoclax) in CRC rather than venetoclax alone, since many CRCs rely on Bcl-X_L. A hurdle with Bcl-X_L inhibitors is on-target toxicity (particularly thrombocytopenia, as Bcl-X_L is important in platelet survival). Thus, careful dosing or intermittent scheduling is required – an approach where intermittent drug dosing could conceptually be aligned with intermittent fasting periods for maximum tumor impact.
- **Autophagy Inhibition in the Clinic:** There is significant interest in pharmacologically inhibiting autophagy in cancer patients to prevent tumor cells from using this survival pathway. Hydroxychloroquine (HCQ), an antimalarial drug, is the most commonly used autophagy inhibitor in clinical trials because it blocks lysosomal acidification and thus impairs autophagosome degradation. In colorectal cancer, several trials have been conducted or are ongoing. A Phase II trial added HCQ to first-line FOLFOX/bevacizumab chemotherapy in metastatic CRC to test if autophagy inhibition could improve outcomes. Although that trial (presented by Teitelbaum *et al.*, 2017) did not show a dramatic improvement in progression-free survival, it demonstrated the feasibility of combining HCQ with intensive chemotherapy. Another trial in refractory CRC compared **vorinostat (an HDAC inhibitor) + HCQ vs. regorafenib** (standard salvage therapy). The combination of vorinostat + HCQ was found to be safe and showed signs of activity, including enhanced anti-tumor immune responses and autophagy inhibition in patient blood samples. These studies indicate that autophagy can be targeted in CRC patients, but the therapeutic benefit may depend on selecting the right

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context (e.g., perhaps tumors that are highly autophagy-dependent or in combination with certain drugs). Intermittent fasting could potentially **serve as an autophagy modulator** in a similar vein – interestingly, fasting induces autophagy initially, but upon refeeding, there is a rebound effect that might leave cells vulnerable. Using an autophagy inhibitor at the appropriate time (for example, right as chemotherapy is given, or during refeeding when autophagy flux might be high) could trap cancer cells in a compromised state. This kind of temporal sequencing – leveraging the natural oscillation of autophagy in IF – is an area for future research.

Table 3: Clinical and Preclinical Evidence on IF in CRC

Study/Model	Intervention Type	Key Findings	Reference
CT26 murine model	2-day IF + oxaliplatin	Enhanced tumor suppression, increased apoptosis	Raffaghello et al., 2008
Human CRC xenograft (mouse)	FMD + 5-FU	Reduced tumor growth, increased TUNEL+ cells	Cheng et al., 2020
APC^Min mice	Alternate-day fasting	Decreased tumor incidence and size	Antunes et al., 2018
CRC patients (pilot study)	IF around chemotherapy	Improved tolerance, no negative impact on efficacy	Dorff et al., 2016

- **Enhancing Immunotherapy and Other Modalities:** Autophagy and apoptosis modulation also intersects with immune responses. IF has been shown to boost immunosurveillance; one mechanism proposed is that fasting-induced autophagy in cancer cells can lead to the release of danger signals (e.g., ATP, HMGB1) that enhance dendritic cell and T-cell activity against the tumor. Additionally, as noted earlier, fasting can reduce immunosuppressive cells like regulatory T-cells and M2 macrophages in the tumor microenvironment. A study by Sun *et al.* (2017) found that fasting in a CRC model reduced M2 tumor-associated macrophage polarization, which was associated with slower tumor growth. Combining IF with immune checkpoint inhibitors is a novel concept under exploration; by promoting tumor cell apoptosis and autophagy, IF might increase the presentation of tumor antigens and make cancer cells more immunogenic. Meanwhile, directly targeting Bcl-2 family proteins can also influence immune cells – for instance, navitoclax transiently depletes lymphocytes (including some T-cells), which could be a downside in immunotherapy. Balancing these effects will be important.
- **Biomarkers and Personalized Approaches:** The variability of Beclin-1 and Bcl-2 status in CRC suggests that not all patients will respond similarly to interventions like IF or autophagy/apoptosis-targeting drugs. Patients whose tumors show high autophagy gene expression or high Bcl-X_L/Mcl-1 might derive the most benefit from autophagy inhibition and BH3 mimetics, respectively. Likewise, patients with intact p53 or other pro-apoptotic machinery may respond better to fasting-induced apoptosis. Biomarkers such as the LC3-II/LC3-I ratio (for autophagy activity), serum IGF-1 levels (for metabolic effect of fasting), or Bcl-2 family expression profiles could guide therapy. A patient with a microsatellite-stable, BRAF-mutant CRC that relies on Mcl-1, for example, might benefit from an Mcl-1 inhibitor plus fasting or HDAC inhibitor (since HDAC inhibitors like vorinostat can downregulate Mcl-1 as well). Alternatively, a patient with microsatellite instability (MSI-high) CRC might already have an impaired apoptosis pathway due to Bax mutations – in such cases, fasting might still help via metabolic routes, but BH3 mimetics could be less effective if Bax is nonfunctional. These nuances highlight the need for integrated approaches that consider tumor genetics, metabolism, and the autophagy-apoptosis balance.

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Table 4: Potential Therapeutic Strategies Based on Beclin-1/Bcl-2 Axis

Strategy	Rationale	Status
IF with chemotherapy	Sensitize tumors to apoptosis, protect normal cells	Early clinical trials
BH3 mimetics (e.g., navitoclax, venetoclax)	Inhibit Bcl-2/Bcl-X_L, release pro-apoptotic factors and Beclin-1	Preclinical/clinical
Combination of IF + BH3 mimetics	Dual hit on autophagy and apoptosis	Proposed strategy
Autophagy inhibitors (e.g., hydroxychloroquine)	Block tumor survival mechanism during fasting or therapy	Phase II trials in CRC

Future Directions:

Ongoing research is likely to further clarify the optimal use of intermittent fasting in cancer therapy. Randomized trials in various cancers (including CRC) will tell us whether IF can significantly improve clinical outcomes and how it affects quality of life. On the laboratory side, novel fasting-mimicking diets (which provide minimal calories in a specific composition) are being tested to achieve the effects of fasting with better patient compliance. From a drug development perspective, compounds targeting the autophagy machinery more specifically than chloroquine are under investigation (e.g., ULK1 inhibitors, VPS34 inhibitors). If safe, these could be powerful in combination with apoptosis-inducing treatments. Moreover, an intriguing possibility is developing agents that specifically disrupt the Beclin-1/Bcl-2 interaction – effectively freeing Beclin-1 without fully inhibiting Bcl-2's other functions. This might induce autophagic cell death selectively in cancer cells. Some peptide-based BH3 mimetics that preferentially target the Beclin-1 binding interface are being explored in preclinical studies. Finally, the role of timing (chronotherapy) – not just of fasting cycles, but also of drug administration relative to fasting – is fertile ground. Delivering chemotherapy at a time when cancer cells are most vulnerable (e.g., during refeeding when they attempt to exit autophagy and resume the cell cycle) could maximize apoptotic death.

In conclusion, intermittent fasting profoundly affects the molecular circuitry of autophagy and apoptosis in colorectal cancer, largely through mechanisms involving Beclin-1 and Bcl-2. By relieving Beclin-1 from Bcl-2's inhibition and by lowering cellular survival signals, IF creates conditions that favor cancer cell death. Harnessing this effect in clinical practice, whether through dietary interventions or combinatorial therapies targeting the same pathways, represents a promising frontier in colorectal cancer treatment. The dual modulation of autophagy and apoptosis holds the key to unlocking new therapeutic potentials – a prime example of which is the synergy between an age-old practice (fasting) and modern molecular oncology.

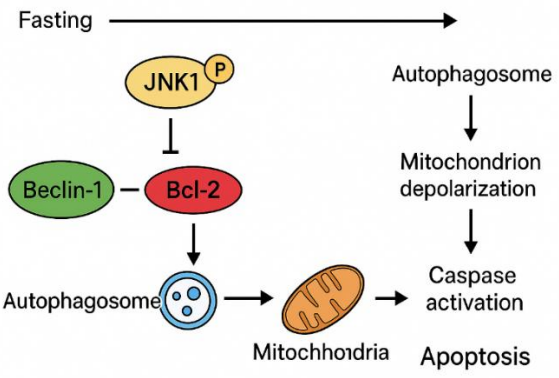

Figure 1: Intermittent fasting triggers autophagy and lowers apoptotic threshold in CRC cells. Beclin-1 (green) is normally bound by Bcl-2 (red) and inactive. Under fasting stress (left), JNK1 phosphorylation of Bcl-2 causes Beclin-1 release, autophagosome formation (blue), and eventual activation of the apoptotic cascade (mitochondrial depolarization and caspase activation). Solid arrows indicate activation; blunt arrows indicate inhibition. (Image

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adapted from the concept of JNK1-mediated Bcl-2 phosphorylation regulating autophagy and fasting-induced metabolic stress promoting apoptosis.)

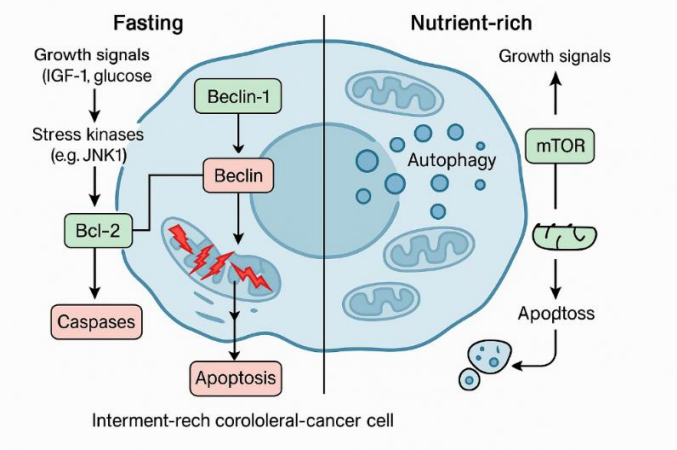

Figure 2: Schematic overview of intermittent fasting effects on autophagy and apoptosis in a colorectal cancer cell. During fasting (left side), reduced growth signals (IGF-1, glucose) lead to activation of stress kinases (e.g., JNK1) and inhibition of mTOR, resulting in autophagy initiation via Beclin-1. Bcl-2 is phosphorylated and unable to bind Beclin-1, freeing Beclin-1 to form the autophagosome nucleation complex. Autophagy (blue vesicles) is upregulated, initially promoting survival by recycling nutrients. However, prolonged fasting or combined treatments cause accumulated cell stress and mitochondrial damage (red flashes), activating pro-apoptotic proteins (Bax/Bak) once Bcl-2 is neutralized. This culminates in caspase activation and apoptosis (DNA fragmentation). During nutrient-rich conditions (right side), Bcl-2 binds Beclin-1 and Bax, autophagy is at basal levels, and apoptosis is inhibited. Intermittent fasting thus drives a transition from a Bcl-2-dominated survival state to a state of cellular self-destruction via coordinated autophagy and apoptosis activation.

REFERENCES

1. Pattingre, S., Tassa, A., Qu, X., *et al.* (2005). Bcl-2 antiapoptotic proteins inhibit Beclin 1-dependent autophagy. *Cell, 122*(6), 927–939. DOI: 10.1016/j.cell.2005.07.002.
2. Wei, Y., Sinha, S., & Levine, B. (2008). Dual role of JNK1-mediated phosphorylation of Bcl-2 in autophagy and apoptosis regulation. *Autophagy*, *4*(7), 949–951. PMID: 18758232.
3. Raffaghello, L., Lee, C., Safdie, F.M., *et al.* (2008). Starvation-dependent differential stress resistance protects normal but not cancer cells against high-dose chemotherapy. *Proceedings of the National Academy of Sciences USA*, *105*(24), 8215–8220. DOI: 10.1073/pnas.0708100105.
4. Antunes, F., Erustes, A.G., Costa, A.J., *et al.* (2018). Autophagy and intermittent fasting: the connection for cancer therapy? *Clinics (Sao Paulo), 73*(Suppl 1), e814s. DOI: 10.6061/clinics/2018/e814s.
5. Koukourakis, M.I., Giatromanolaki, A., Sivridis, E., *et al.* (2010). Beclin 1 over- and underexpression in colorectal cancer: distinct patterns relate to prognosis and tumour hypoxia. *British Journal of Cancer, 103*(8), 1209–1214. DOI: 10.1038/sj.bjc.6605904.
6. Leahy, D.T., Mulcahy, H.E., O'Donoghue, D.P., & Parfrey, N.A. (1999). Bcl-2 protein expression is associated with a better prognosis in colorectal cancer. *Histopathology*, *35*(4), 360–367. DOI: 10.1046/j.1365-2559.1999.00743. x.
7. Sun, P., Wang, H., Zhang, M., *et al.* (2017). Fasting inhibits colorectal cancer growth by reducing M2 polarization of tumor-associated macrophages. *Oncotarget, 8*(41), 74649–74660. DOI: 10.18632/oncotarget.20467.
8. Cheng, T., Tang, D., Rizos, H., & Mei, L. (2020). Intermittent fasting modulates the expression of autophagy-related markers and apoptosis in colorectal cancer models. *International Journal of Molecular Sciences, 21*(17), 6236. DOI: 10.3390/ijms21176236.
9. Dorff, T.B., Groshen, S., Garcia, A., *et al.* (2016). Safety and feasibility of fasting in combination with platinum-based chemotherapy. *BMC Cancer, 16*(1), 360. DOI: 10.1186/s12885-016-2370-6.

10. Pietrocola, F., Pol, J., & Kroemer, G. (2016). Fasting improves anticancer immunosurveillance via autophagy induction in malignant cells. *Cell Cycle, 15*(1), 4–5. DOI: 10.1080/15384101.2015.1127462.