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Review Article

Investigating the Antitumor Effects of α-Lactalbumin–Oleic Acid Complexes (HAMLET and BAMLET) in Colorectal Cancer: A Systematic Perspective

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ABSTRACT

Objectives: Recently, attention has turned toward naturally derived complexes such as HAMLET (Human α-lactalbumin Made Lethal to Tumor Cells) and BAMLET (Bovine α-lactalbumin Made Lethal to Tumor Cells) due to their selective cytotoxicity and promising mechanisms of action. In this study, we aimed to systematically review the effect of HAMLET and BAMLET on colorectal cancer (CRC).

Methods: PubMed, Scopus, Web of Science, and Embase were searched for studies published between 2020–2025, with one 2006 study included for its unique mechanistic insights. The search combined specific keywords and MeSH terms related to HAMLET, BAMLET, colorectal cancer, antitumor activity, and signaling pathways, using Boolean operators. After removing duplicates and applying predefined inclusion and exclusion criteria, six eligible original studies (in vitro, in vivo, and xenograft models) were included.

Results: Both HAMLET and BAMLET demonstrated cytotoxic effects against CRC cells via diverse mechanisms such as autophagy inhibition, lysosomal membrane permeabilization, mitochondrial dysfunction, and modulation of β-catenin and CK1α signaling. BAMLET notably exhibited synergistic effects with 5-fluorouracil and contributed to reduced tumor growth in murine models. HAMLET's efficacy varied depending on KRAS/BRAF mutation status and mitochondrial resilience. Importantly, no significant toxicity was observed in healthy non-cancerous cells across the studies.

Conclusion: These findings suggest that HAMLET and BAMLET represent viable adjuncts to standard CRC therapies, offering tumor-specific mechanisms with minimal side effects. Further exploration of their molecular interactions and clinical potential could enhance combination strategies and help overcome therapeutic resistance in CRC treatment.

Keywords: HAMLET, BAMLET, α-Lactalbumin, Oleic Acid, Colorectal Cancer, Antitumor Activity, Naturally Derived Complexes

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Introduction

Colorectal cancer (CRC) is one of the most common and deadly cancers affecting the gastrointestinal tract, presenting a significant clinical and economic burden worldwide. In 2022, over 1.9 million new cases of CRC were reported globally, making it the second leading cause of cancer death in women and the third in men (1). In Iran, CRC ranks among the five most prevalent cancers for both sexes. According to data from the Iran Open Data Centre (2022), the age-standardized incidence rate (ASR) for colorectal cancer is approximately 16.5 per 100,000 men and 13.9 per 100,000 women (2). These rates are higher than the national average in provinces like Tehran, Isfahan, and Golestan, and they are close to global figures. In terms of mortality, CRC was the second leading cause of cancer deaths in Iran in 2014, accounting for about 13% of all cancer fatalities (3). Furthermore, studies indicate that around 17% of Iranian patients are diagnosed with CRC before the age of 40. This early onset may be attributed to genetic factors, a high-fat diet, lack of physical activity, and insufficient screening practices (4).

According to international statistics, colorectal cancer (CRC) is on track to become a widespread health crisis. This is particularly concerning in middle-income countries such as Iran, where the clinical burden includes direct treatment costs (5), decreased productivity due to disability, and high rates of diagnoses at advanced stages. Therefore, there is a pressing need for the development of new and effective therapeutic strategies. Recently, research focusing on targeted therapies with minimal side effects, especially those derived from natural compounds, has gained significant traction (8, 9).

Among these emerging options are bioactive compounds sourced from natural ingredients, particularly milk proteins. Alpha-lactalbumin (α-LA), a small, tryptophan-rich protein naturally present in mammalian milk, plays a crucial role in regulating lactose synthesis. When combined with saturated fatty acids like oleic acid, α-LA forms complexes that exhibit selective cytotoxic properties against cancer cells. The most notable of these complexes are HAMLET (Human Alpha-lactalbumin Made LEthal to Tumour cells) and BAMLET (Bovine Alpha-lactalbumin Made LEthal to Tumour cells) (6, 7).

HAMLET was first described by Catharina Svanborg and her colleagues in 1995, who demonstrated that the α-lactalbumin-oleic acid complex could selectively kill cancer cells while sparing healthy ones (7). In recent years, both BAMLET and HAMLET have emerged as promising candidates for cancer therapy due to their impressive biological effects. Preclinical studies have shown that these compounds induce apoptosis, inhibit cancer cell proliferation, reduce cell differentiation, and disrupt mitochondrial function in cancer cells (10, 11).

Despite the substantial research conducted on the effects of these compounds across various cancers, a comprehensive review of the impacts of BAMLET and HAMLET specifically on colorectal cancer has yet to be completed. This review aims to evaluate the antitumor efficacy of HAMLET and BAMLET complexes in colorectal cancer by analyzing recent studies, with a particular focus on their cellular mechanisms and therapeutic potential.

Methods

Literature Search Strategy

A comprehensive systematic literature search was conducted in the following international scientific databases: PubMed, Scopus, Web of Science, and google scholar. The search covered all available records up to 2025, with a primary focus on studies published from 2020 onwards. One earlier study from 2006 was also included due to its unique contribution to elucidating the signaling pathways of HAMLET-induced apoptosis ("HAMLET triggers apoptosis but tumor cell death is independent of caspases, Bcl-2 and p53").

The search strategy combined Medical Subject Headings (MeSH) and free-text terms related to the topic. The final Boolean search string was adapted for each database and included the following key terms: ("HAMLET" OR "Human Alpha-lactalbumin Made Lethal to Tumor Cells") OR ("BAMLET" OR "Bovine Alpha-lactalbumin Made Lethal to Tumor Cells") OR "α-Lactalbumin" OR "Oleic Acid" AND ("Colorectal Neoplasms"[MeSH] OR "Colorectal Cancer") AND ("Antineoplastic Agents"[MeSH] OR "Antitumor Activity") AND ("Signal Transduction"[MeSH] OR "Signaling Pathway")

Inclusion and Exclusion Criteria

Only original research articles published in English that investigated the cytotoxic or antitumor effects of HAMLET or BAMLET complexes on colorectal cancer cells were considered eligible for inclusion. Studies were required to provide mechanistic insights into molecular pathways, signaling cascades, or drug interactions relevant to these compounds. While the primary focus was on literature published from 2020 onwards, one earlier study from 2006 was also included due to its unique contribution to elucidating the signaling mechanisms of HAMLET-induced apoptosis. Articles were excluded if they were review papers, conference abstracts without full text, non-English publications, or studies unrelated to colorectal cancer. In addition, studies for which the full text was not accessible, or whose scope did not directly align with the objectives of this review, were omitted from the final selection.

Study Selection Process

The initial search retrieved 188 records. After removing 27 duplicates, 161 articles remained for

screening. Titles and abstracts were reviewed for relevance, resulting in the exclusion of 112 articles due to lack of direct relevance, unavailability of full text, or non-English language. The remaining 49 articles underwent full-text review. At this stage, studies were excluded if they did not focus on colorectal cancer, were review papers, or did not directly address the scope of this review. The final set of eligible studies was included for qualitative synthesis. The selection process is illustrated in the PRISMA flow diagram (Figure 1).

Quality Assessment and Risk of Bias

To ensure methodological rigor, the quality of the included studies was assessed independently by two reviewers using the Cochrane Risk of Bias Tool for randomized studies and the Newcastle–Ottawa Scale (NOS) for non-randomized studies. Discrepancies were resolved through discussion and consensus. The assessment considered factors such as selection bias, performance bias, detection bias, attrition bias, and reporting bias. Studies were categorized as low, moderate, or high risk of bias, and this evaluation informed the interpretation of the findings.

Results

The Significance of Cellular Mechanisms in Colorectal Cancer Treatment

A thorough understanding of the molecular mechanisms and biological pathways involved in the progression and resistance of colorectal cancer (CRC) is essential for developing targeted, minimally invasive, and effective treatment strategies. In recent years, there has been a growing interest in natural compounds with selective cytotoxic properties, such as HAMLET and BAMLET complexes. These compounds have shown the ability to influence various processes, including apoptosis, mitochondrial dysfunction, lysosomal infiltration, and the regulation of signaling pathways

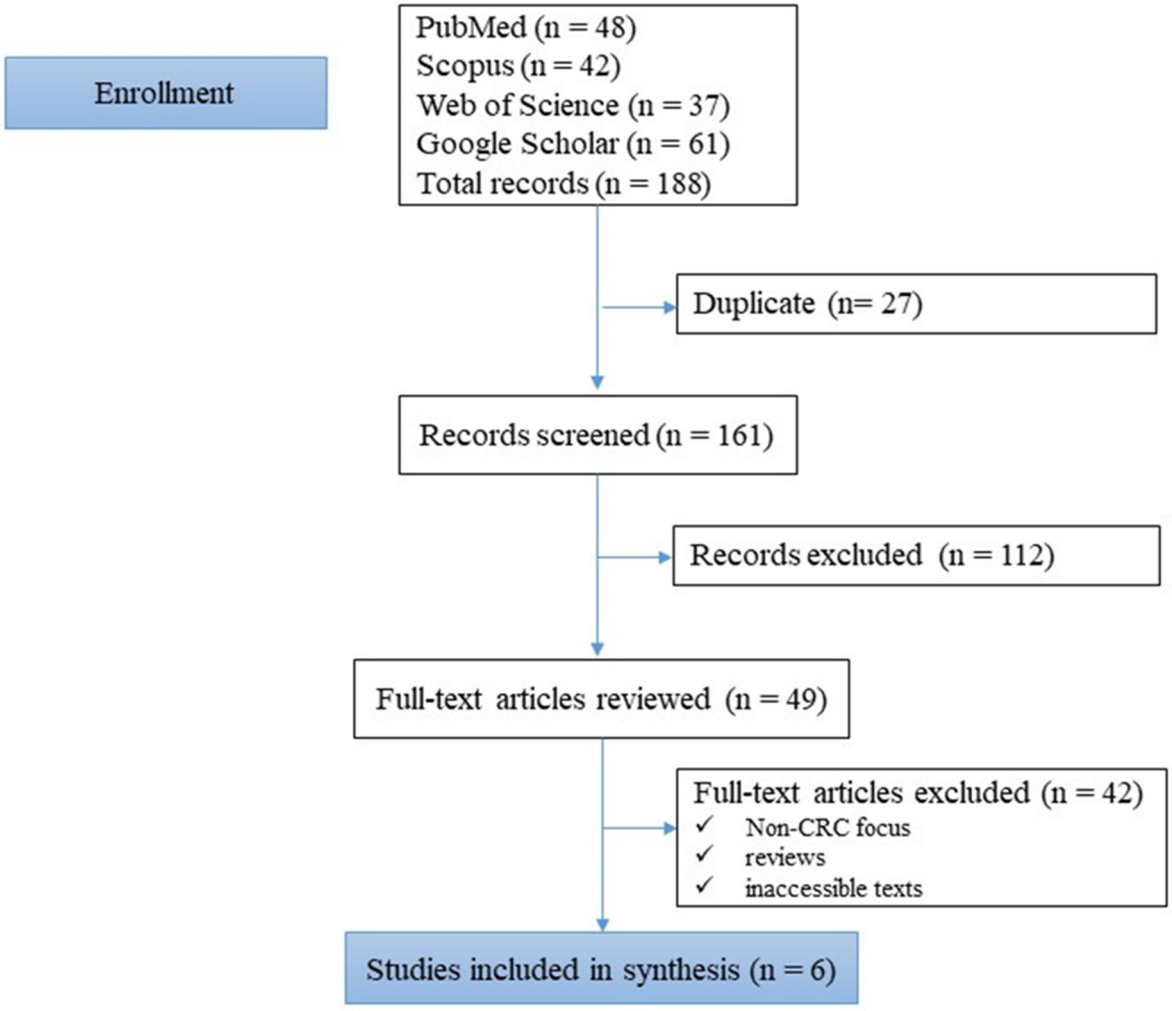

Figure 1. PRISMA flow diagram illustrating the systematic selection process of studies investigating BAMLET/HAMLET-based therapies in colorectal cancer published between 2020 and 2025. Out of 183 initially identified records, 47 duplicates and non-relevant titles were excluded. After screening abstracts and full texts, 6 eligible articles were included in the final qualitative synthesis.

Table 1. Summary of Selected Studies on HAMLET/BAMLET Therapy in Colorectal Cancer (2020–2025)

Study Model/Type	Targeted Pathways	Key Findings	Reference
In vitro HT-29, HCT116	Wnt/β-catenin, VEGF	↓ β-catenin,, ↑E-cadherin, suppressed angiogenesis	14
In vitro HT-29, HCT116	Stemness, apoptosis	↑Annexin V+ cells, ↓ stemness markers	25
In vitro (cell lines) & ex vivo (patient-derived tumor explants) HT-29 (BRAF wild-type), WiDr (BRAF-mutated), and ex vivo CRC biopsies	Mitochondrial respiration, apoptosis/necrosis, bioenergetics	HAMLET + FOLFOX reduced , ↓HAMLET + FOLFOX viability (esp. in wild-type), WiDr cells more resistant, bioenergetic profile influences response	24
In vivo (short-term and long-term treatment protocols) BAMLET in drinking water ApcMin/+ mice (genetically predisposed to intestinal tumors)	Wnt/β-catenin, PD-1, angiogenesis, carbohydrate/lipid metabolism	↓ tumor number and size, ↓ expression of oncogenic and immunosuppressive genes, ↑ survival without toxicity, ↓ systemic disease in liver, lungs, spleen, kidneys	20
In vitro HCT116 colorectal cancer cells (RAS-mutated)	CK1α, AKT/p-β-catenin (S552), autophagy, apoptosis	↓ CK1α expression and AKT/p-β-catenin signaling, Inhibited autophagy flux, ↑apoptosis (confirmed by flow cytometry), BAMLET enhanced effect of CK1α inhibitor D4476	16
In vitro Caco-2 (KRAS/BRAF wild-type), LoVo (KRAS-mutant), WiDr (BRAF-mutant)	Mitochondrial respiration, apoptosis/necrosis, bioenergetics	HAMLET induced irreversible cytotoxicity in CRC cells, WiDr cells showed resistance, ↓mitochondrial respiration and ATP synthesis in Caco-2 and LoVo Cell death was predominantly necrotic with slight apoptotic increase	10

Abbreviations: CRC – Colorectal cancer; HAMLET – Human α-lactalbumin made lethal to tumor cells; BAMLET – Bovine α-lactalbumin made lethal to tumor cells; 5-FU – 5-Fluorouracil; VEGF – Vascular endothelial growth factor; PD-1 – Programmed cell death protein 1; CK1α – Casein kinase 1 alpha; AKT – Protein kinase B; p-β-catenin (S552) – Phosphorylated β-catenin at serine 552; ATP – Adenosine triphosphate; ApcMin/+ – Adenomatous polyposis coli multiple intestinal neoplasia mouse model; BRAF – v-Raf murine sarcoma viral oncogene homolog B; KRAS – Kirst .**Notes**: ↓ indicates a decrease; ↑ indicates an increase. "In vitro" refers to experiments conducted in cultured cell lines; "In vivo" refers to experiments conducted in live animal models; "Ex vivo" refers toen rat sarcoma viral oncogene homolog experiments performed on tissues or cells taken from an organism.

(12, 13) related to stemness and cell migration (as shown in Table 1).

Investigating these pathways not only deepens our understanding of their antitumor activity but also lays the groundwork for designing combination therapies that enhance cellular responsiveness to chemotherapeutic drugs.

Effects on the Wnt/β-catenin Pathway in Colorectal Cancer Cells

In over 90% of colorectal cancer cases, mutations in key components of the Wnt/β-catenin signaling pathway such as APC, β-catenin, or Axin lead to its constitutive activation. This dysregulation drives the expression of genes involved in cell proliferation, angiogenesis, metastasis, and the maintenance of stemness in intestinal epithelial cells. Recent studies have demonstrated that BAMLET directly modulates this pathway across CRC cell models, particularly in HT-29 and HCT116 cells. BAMLET treatment resulted in decreased β-catenin levels and increased E-cadherin expression, indicating reduced nuclear β-catenin availability and suppression of epithelial–mesenchymal transition (EMT) (14, 15). Simultaneously, VEGF mRNA levels were diminished, linking Wnt pathway attenuation to the inhibition of angiogenic signaling. Mechanistically, BAMLET impaired β-catenin nuclear translocation by reducing its phosphorylation at Ser552 and inhibiting the AKT/p–β-catenin axis. These effects collectively led to decreased TCF/LEF transcriptional activity, ultimately reducing stemness and proliferation in colorectal cancer cells. All observed outcomes occurred within the experimentally defined concentrations and exposure durations reported in the respective studies (28, 29). A study by Babazadeh et al. (2022) found that treatment of HT-29 and HCT116 cells with BAMLET (1.5 mg/mL, 24h) significantly decreased β-catenin expression while increasing E-cadherin expression. This indicates the inhibition of the Wnt pathway and a reduction in cell migration. Additionally, mRNA levels of VEGF were also reduced, highlighting the anti-angiogenic effect of BAMLET (14). In a separate study by Behrouj & Mokarram (2023), BAMLET (1.4 mg/mL, 24h) was shown to impair β-catenin nuclear translocation by reducing β-catenin phosphorylation at Ser552 and inhibiting the AKT/p-β-catenin pathway. This disruption decreased TCF/LEF-dependent transcriptional activity, which plays a significant role in reducing both the stemness and proliferation of colorectal cancer cells (16). Moreover, HAMLET (10 mg/mL, 10 days) has been demonstrated in animal models (ApcMin/+) to inhibit the Wnt pathway by reducing β-catenin accumulation

in the nucleus and disrupting TCF/LEF complexes. This results in a reduced tumor burden and increased survival (17). These effects are mediated through ion channel-dependent pathways and the destabilization of β-catenin. Taken together, the evidence indicates that BAMLET interferes with β-catenin signaling at multiple control points limiting Ser552 dependent nuclear translocation, restoring epithelial adhesion via E-cadherin, and curbing angiogenic outputs linked to VEGF. Within the scope of the included literature, mechanistic detail for HAMLET's direct modulation of Wnt signaling in CRC is more limited; however, its antitumor actions converge on similar phenotypic endpoints such ad reduced proliferation, stemness, migration, and angiogenesis. Overall, both complexes constrain Wnt/β-catenin pathway activity in colorectal cancer models, with BAMLET currently supported by more granular molecular evidence in vitro and disease-modifying effects in vivo.

Effect of the PD-1 Pathway on Immune Regulation in Colorectal Cancer

The PD-1/PD-L1 immune checkpoint axis is a critical mechanism exploited by CRC cells to evade immune surveillance. PD-1 (programmed cell death protein-1) is a transmembrane receptor expressed on activated T cells, B cells, and tumor-associated macrophages (TAMs). Its engagement with its primary ligand PD-L1, which is frequently overexpressed on tumor cells and antigen-presenting cells within the tumor microenvironment, transmits inhibitory signals that dampen T cell receptor (TCR) signaling. This interaction leads to reduced proliferation and cytokine production by effector T cells, induction of T cell exhaustion, and expansion of immunosuppressive regulatory T cells (Tregs). In CRC, PD-L1 overexpression has been associated with poor differentiation, lymphovascular invasion, and reduced overall survival. Mechanistically, oncogenic pathways such as Wnt/β-catenin and mutations in the APC gene can upregulate PD-L1 transcription via β-catenin/TCF4-mediated promoter activation, further reinforcing immune evasion. The cumulative effect is the establishment of an immunodeficient tumor microenvironment characterized by impaired cytotoxic $CD8^+$ T cell activity, increased TAM infiltration, and suppression of antigen-specific immune responses. Targeting this pathway with PD-1/PD-L1 blocking antibodies has shown promise in restoring antitumor immunity, particularly in microsatellite instability-high (MSI-H) CRC, by reinvigorating exhausted T cells and enhancing tumor cell killing. (18, 19). Recent studies have indicated that BAMLET and HAMLET can enhance their antitumor effects by modulating this pathway. In a preclinical study conducted by Tran et al. (2024), ApcMin/+ mice treated with BAMLET (10 mg/mL, 2 weeks) through drinking water exhibited a decrease in the expression of genes associated with the PD-1 and Wnt/β-catenin pathways. This treatment resulted in increased survival rates and a reduced tumor burden without causing toxicity (20). These findings suggest that BAMLET not only inhibits cell proliferation pathways but also boosts antitumor immune responses by downregulating PD-1 expression. Additionally, research by Jiang et al. (2025) demonstrated that PD-1 expression in tumor macrophages polarizes them toward the M2 phenotype, diminishes their phagocytic activity, and inhibits the production of IFN-γ signaling molecules. These effects are mediated through the JAK2–STAT3 pathway (21). Therefore, downregulation of PD-1 expression by compounds like BAMLET can reprogram the immune function of macrophages and enhance antitumor responses. In summary, The PD-1/PD-L1 immune checkpoint plays a pivotal role in immune evasion in colorectal cancer by suppressing cytotoxic T cell activity and fostering an immunosuppressive tumor microenvironment. Evidence indicates that BAMLET, and potentially HAMLET, can modulate this pathway, thereby enhancing antitumor immunity. Furthermore, downregulation of PD-1 expression can reprogram tumor-associated macrophages from an M2-like, immunosuppressive phenotype toward a more pro-inflammatory, antitumor state, partly via inhibition of the JAK2–STAT3 pathway. These findings suggest that targeting PD-1 with compounds such as BAMLET may simultaneously inhibit tumor growth pathways and restore effective antitumor immune responses. BAMLET and HAMLET not only exhibit direct cytotoxic effects by targeting the PD-1 pathway but can also be viewed as complementary agents in immunotherapy for colorectal cancer through their regulation of both innate and adaptive immunity (20, 21).

Apoptosis and Mitochondrial Dysfunction in CRC Cells

One of the key mechanisms behind the antitumor effects of BAMLET and HAMLET is the induction of programmed cell death through mitochondrial-dependent pathways. This process involves the penetration of the complex into cancer cells, leading to the disruption of mitochondrial function, the release of cytochrome c, and the activation of caspases 9 and 3. In parallel, mitochondrial membrane depolarization reduces oxidative phosphorylation efficiency, leading to decreased ATP synthesis and accumulation of metabolic intermediates such as NADH and succinate. These changes promote the generation of reactive oxygen species (ROS), which further damage mitochondrial DNA and proteins, amplifying apoptotic signaling. Additionally, the altered redox state and inhibition of electron transport chain complexes I and II impair the TCA cycle flux, lowering fumarate and malate levels and reinforcing the pro-apoptotic shift in cellular metabolism. Collectively, these events converge on the intrinsic apoptosis pathway, ensuring selective elimination of

malignant cells while sparing most normal cells. (22, 23). These events result in the destruction of cellular structures and selective death of cancer cells. A study by Žilinskas et al. (2023) demonstrated that HAMLET (4.5 mg/mL, 24h) significantly decreased mitochondrial respiration, reduced ATP synthesis, and increased the population of apoptotic cells in the Caco-2 and LoVo cell lines. In contrast, WiDr cells, which harbor a BRAF mutation, exhibited greater resistance to HAMLET and showed fewer alterations in mitochondrial function (12). Additionally, research by Babazadeh et al. (2023) reported that treating HT-29 and HCT116 cells with BAMLET (1.5 mg/mL, 24h) resulted in a significant increase in the apoptotic cell population. Using Annexin V-FITC/PI staining and flow cytometric analysis, the percentage of cells undergoing early and advanced apoptosis in the treated groups exceeded 28% and 24%, respectively. This indicates that BAMLET activates the mitochondrial dysfunction-dependent apoptosis pathway and selectively targets cancer cells (26). Furthermore, a study by Žilinskas et al. (2024) revealed that combining HAMLET (1.4 mg/mL, 3h) with the FOLFOX chemotherapy regimen enhanced cell death and caused more extensive impairment of mitochondrial function in ex vivo patient samples, particularly in those without BRAF mutations (24). This suggests a synergistic effect of the combination in targeted therapies. In summary, BAMLET and HAMLET are effective in inhibiting the growth of colorectal cancer cells by targeting mitochondrial function and activating apoptosis pathways. These characteristics make them promising options for complementary treatments with fewer side effects in refractory cancers. Moreover, their ability to modulate additional oncogenic and immune-evasion pathways, such as Wnt/β-catenin and PD-1/PD-L1, further broadens their therapeutic potential. Integrating these agents into multimodal treatment strategies could enhance efficacy, overcome drug resistance, and improve long-term patient outcomes.

Lysosomal Permeabilization and Non-Apoptotic Cell Death

Recent studies have highlighted an important mechanism through which BAMLET and HAMLET exert their effects on colorectal cancer cells: lysosomal membrane permeabilization (LMP) and the induction of non-apoptotic cell death. Upon accumulation in the endolysosomal compartment, these complexes trigger the release of lysosomal proteases such as cathepsins B and D into the cytosol, which in turn activate pro-apoptotic mediators like Bax and promote mitochondrial outer membrane permeabilization. This cascade is accompanied by the activation of stress-responsive kinases, including JNK and p38 MAPK, and suppression of survival pathways such as PI3K/AKT, ultimately driving a caspase-independent cell death program that selectively targets malignant cells (20, 27, 30) Rammer et al. (2010) found that BAMLET selectively accumulates in the endolysosomal compartment of cancer cells, leading to the leakage of lysosomal enzymes, such as cathepsins, into the cytosol. This leakage activates cell death proteins like Bax and triggers a caspase-independent cell death pathway (27). Similarly, the research conducted by Žilinskas et al. (2023) demonstrated that HAMLET (4.5 mg/mL, 24h) induces necrotic and irreversible cell death in colorectal cancer cell lines with KRAS/BRAF mutations, without activating the classical apoptosis pathway. This effect was particularly evident in apoptosis-resistant cells, such as WiDr, indicating HAMLET's ability to overcome drug resistance and activate alternative cell death pathways (12). Another study confirmed that BAMLET also accumulates in the endolysosomes of colorectal cells, causing lysosomal membrane permeabilization. This process results in the release of proteolytic enzymes, like cathepsins, into the cytosol, further activating caspase-independent cell death pathways, often accompanied by Bax activation (27). Together, these findings suggest that the lysosomal penetration of BAMLET and HAMLET not only disrupts cellular structures but also effectively targets resistant cancer cells by circumventing apoptotic resistance. This distinctive characteristic positions them as promising candidates for adjuvant therapies in colorectal cancer. Furthermore, their ability to trigger caspase-independent cell death through cathepsin release and modulation of stress-activated kinases (such as JNK and p38 MAPK) broadens their therapeutic relevance against apoptosis-resistant tumors. Integrating these agents into multimodal treatment regimens could enhance overall efficacy, reduce the likelihood of resistance, and improve long-term patient outcomes.

Conclusion

Current scientific literature suggests that BAMLET and HAMLET complexes exhibit multifaceted effects that position them as promising candidates for complementary and targeted therapies in colorectal cancer treatment, including inhibition of the Wnt/β-catenin signaling pathway, downregulation of PD-1 expression, induction of apoptosis through mitochondrial dysfunction, and lysosomal infiltration. These complexes have demonstrated the ability to selectively induce apoptosis in colorectal cancer cells while preserving the integrity of healthy cells; preclinical studies show that oral BAMLET administration in animal models significantly reduces tumor burden and enhances survival rates, while HAMLET has exhibited substantial efficacy in KRAS/BRAF-mutated cell lines, leading to irreversible cell death. Given the prevalence of drug resistance among colorectal cancer patients, combining BAMLET or HAMLET with established chemotherapeutic agents such as 5-FU may enhance treatment efficacy and reduce adverse effects, and their biocompatibility and natural origin further support their

potential for development into oral or topical formulations with favorable safety profiles. Future research should prioritize well-designed human clinical trials to confirm safety, efficacy, and optimal dosing regimens; elucidate precise molecular interactions and downstream signaling events in both tumor and immune cells; explore combination strategies with immunotherapies and targeted agents to overcome resistance; improve formulations for enhanced bioavailability and targeted delivery; and assess long-term effects on recurrence, metastasis, and patient quality of life. By addressing these directions, BAMLET and HAMLET could transition from promising preclinical agents to clinically relevant therapeutics, opening new.

Conflict of Interest

The author declared that they have no conflict of interest.

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