Food Science of Animal Products

Review Article

Role of food protein-derived peptides in the treatment of colon cancer

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Abstract: Colon cancer is one of the leading causes of cancer-related deaths and the second most common cancer in Western countries. Despite the availability of various treatment modalities, these therapies have not been fully successful due to their limited effectiveness and serious side effects. As a result, an increasing number of researchers are focusing on the search for natural active substances as potential agents for the treatment and prevention of colon cancer. Bioactive peptides derived from proteins play an important role in maintaining body functions. Several *in vivo* and *in vitro* studies have demonstrated that protein peptides can help prevent and mitigate colon cancer. This paper, therefore, explores the mitigating effects and mechanisms of action of protein peptides from different sources on colon cancer, as well as the factors influencing their anticancer effects. It provides a scientific basis for the prevention and treatment of colon cancer and for the development of new raw materials for foods with potential anticancer properties.

Keywords: protein; hydrolysate; peptide; colon cancer

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1 Introduction

Colon cancer is prevalent in developed nations and poses a significant public health challenge. Colorectal cancer is the second most common cause of cancer-related deaths globally and ranks as the third most common type of cancer[1]. According to Cancer Statistics 2024, colorectal cancer ranked fourth in cancer deaths among individuals under 50 in the late 1990s, but it now ranks first among men and second among women[2]. The development of colon cancer is affected by genetic and epigenetic factors, as well as certain non-genetic factors that affect colon environment homeostasis[3]. Major non-genetic factors include excessive red meat consumption, low intake of fruits and vegetables, poor diet, age, alcohol abuse, obesity, low physical activity, and smoking[4–6]. The conventional treatment options for colon cancer generally include surgery, chemotherapy, and targeted therapy[7]. However, the overall survival of patients with multiple metastases is often reduced to a few months due to the toxic side effects and ineffectiveness of certain drugs[8–9]. Additionally, some elderly patients are not suitable for surgery due to high postoperative complications and mortality rates[10]. Current clinical treatments are often ineffective and prone to drug resistance and toxic side effects, limiting the effectiveness of colon cancer therapy. Thus, there is an urgent need for new, efficient, and low-toxicity anti-cancer drugs.

In recent years, protein hydrolysates and peptides have garnered significant attention due to their safety, low cost, and numerous health benefits, positioning them as important raw materials for the development of functional foods and nutraceuticals. Protein hydrolysates can be produced through the chemical or enzymatic hydrolysis of proteins into peptides of varying sizes[11]. *In vitro* enzymatic hydrolysis commonly utilizes proteolytic enzymes from animals (e.g., pepsin, trypsin, chymotrypsin), plants (e.g., papain, ficin), or microorganisms (e.g., alcalase, neutrase, Protamex™)[12]. These peptides, typically consisting of two to thirty amino acids, exhibit bioactivity that is largely determined by their amino acid composition and sequence[13]. Generally, peptides of smaller molecular weight are more digestible, bioavailable, and frequently exhibit higher biological activity than intact proteins[14]. Numerous studies have investigated the bioactivities of peptides from various sources, revealing a wide range of functional properties, including anticancer, antihypertensive, antimicrobial, anti-obesity, antioxidant, antithrombotic, immunomodulatory, and opioid effects[15–18]. Of particular interest, recent research has increasingly highlighted the therapeutic and preventive potential of protein-derived peptides in the treatment of colon cancer. These anticancer peptides are predominantly derived from plants, animals, seafood, and microorganisms. For instance, peptides derived from legumes have been shown to effectively inhibit the proliferation of Caco-2, HT-29, and HCT-116 colon cancer cells[19]; silkworm pupa protein (SPP) has been found to reduce the metabolism of DLD-1 cells and promote apoptosis[20]; peptides from mackerel proteins have demonstrated inhibitory effects on the growth of SW742 cells[21]; and certain bacteriocins, such as colicin, nisin, and microcin, have also been shown to suppress the progression of colon cancer.

This review discusses the alleviating effects of protein-derived peptides from various sources on colon cancer, including mechanisms such as the promotion of apoptosis, enhancement of antioxidant activity, inhibition of the renin-angiotensin system (RAS), anti-inflammatory effects, and inhibition of cancer cell metastasis, as well as the factors influencing their biological activity. The anti-colon cancer potential of these peptides supports the development of functional foods containing protein-derived peptides.

2 Alleviating effects of protein-derived peptides from different sources on colon cancer

2.1 Alleviating effects of plant-based protein-derived peptides on colon cancer

Purafect® and Esperase® enzymes were used to hydrolyze fenugreek seed protein to prepare a protein hydrolysate. After treating undifferentiated Caco-2/TC7 cells with 1 mg/mL of protein hydrolysate, cell viability decreased. Compared to the untreated group, the inhibition rate of Purafect-treated protein peptides increased from 27% at 24 h to 55% at 72 h[22]. Using different strains of soybeans, protein isolates were prepared at alkaline pH and hydrolyzed using alkaline protease to produce peptide hydrolysates. Treatment of cells with peptide fractions from N98-4445A and S03-543CR high oleic soybean lines showed a 73% inhibition of HCT-116 cell growth[23]. A study investigated the antiproliferative effect of peptides from the non-digestible fraction (NDF) of common beans after simulating gastrointestinal digestion *in vitro* on human colorectal cancer cells. The results showed that after treating cells with four types of bean NDF peptide extracts for 24 h, cell proliferation decreased in a concentration-dependent manner. Among the four varieties, three NDF peptide extracts (Azufrado Higuera, Bayo Madero, and Negro 8025) exhibited antiproliferative effects on HCT-116 and RKO cells[24]. Lunasin, a natural peptide extracted from soybeans, exhibited significant cytotoxic effects on HT-29 cells, with a twofold increase in the percentage of apoptotic cells after lunasin treatment[25]. Walnuts were chosen as the experimental raw material, and pancreatic chymotrypsin, trypsin, and microbial enzyme K were used to enzymatically digest the proteins in walnut kernels. The peptide fractions produced after enzymatic digestion showed significant inhibitory effects on HT-29 cells, with the inhibition rate reaching (51 ± 1.45)%[26]. Corn pollen protein (CPP), as a non-hydrolyzed protein, usually does not exhibit antiproliferative activity. However, when CPP was hydrolyzed by pepsin to produce peptides, it exhibited significant inhibitory effects on the viability of HT-29 colon cancer cells in a concentration-dependent manner. At a peptide concentration of 5 mg/mL, cell viability was reduced to 32%[27]. BG-4 is a new peptide isolated, purified, and identified from bitter gourd. BG-4 exhibited significant inhibitory effects on two types of colon cancer cells, HCT-116 and HT-29 cells. In this experiment, it was found that when BG-4 was applied to these cancer cells for 48 h, its 50% inhibitory concentration (IC_{50}) were 134.4 and 217.0 μg/mL, respectively. Further studies revealed that a significant increase in apoptosis in HCT-116 and HT-29 colon cancer cells was observed at a BG-4 concentration of 125 μg/mL after 16 h of treatment. After treatment, the percentage of apoptotic cells increased from 5.4% to 24.8% for HCT-116 cells and from 8.5% to 31.9% for HT-29 cells. This is the first report on the anticancer potential of a novel bioactive peptide isolated from bitter gourd *in vitro*, supporting the potential therapeutic properties of BG-4 for colon cancer[28].

Using germinated soybeans as raw materials, soy protein concentrate was hydrolyzed by pepsin/trypsin, and different peptide fractions were obtained by ultrafiltration. The results showed that peptides from germinated soy protein inhibited the proliferation of Caco-2, HT-29, and HCT-116 cells in a dose-dependent manner during simulated gastrointestinal digestion. Higher molecular weight peptides (> 10 and 5–10 kDa) were more effective in inhibiting colon cancer cells than smaller molecular weight peptides (< 5 kDa)[19]. A study investigated the anticancer activity of hydrolysates from riceberry rice bran protein (HRBE) obtained by alkaline endopeptidase hydrolysis on colon cancer cell lines. MTT assay results showed that normal cells (PCS-201-010) had a lower toxic response to this hydrolysate compared to colon cancer cells. The hydrolysates were more toxic to SW-620 cells than HT-29 cells, with IC_{50} of 5 468 μg/mL for SW-620 cells and 6 054 μg/mL for HT-29 cells. Moreover, compared to other fractions, the > 50 kDa fraction exhibited better growth inhibitory activity on SW-620 cells. Staining with senescence-associated β-galactosidase (SA-β-gal) revealed that the percentage of cells expressing SA-β-gal was as high as 86%, compared to 32% in SW-620 cells[29]. In another experiment, a 5 kDa peptide fraction isolated from rice bran protein hydrolysate also exhibited effective inhibitory effects on colon cancer cells. After treatment with 500 μg/mL of the peptide fraction, the proliferation of HCT-116 cells was inhibited with an IC_{50} of approximately 750 μg/mL[30]. A 35 kDa protein (named FMBP) was extracted and purified from foxtail millet bran water extract. FMBP exhibited time- and dose-dependent growth inhibition of colon cancer cell lines DLD-1, SW480, and HT-29, while showing no negative effects on normal human colon epithelial cells[31]. Sweet potato proteolytic hydrolysate (SPPH) was obtained by treatment with different proteases, and its inhibitory effect on HT-29 cells was evaluated. SPPH exhibited inhibitory effects on the proliferation of HT-29 cells, with the SPPH obtained using alkaline protease showing the highest inhibitory effect and the lowest IC_{50} of 119.72 μg/mL. The < 3 kDa fraction exhibited the strongest antiproliferative activity and significantly inhibited cell migration[32]. Peptide fractions from rice bran proteins, including those with a molecular weight of 5 kDa, significantly inhibited the growth of colon cancer cells (Caco-2 and HCT-116) in the dose range of 600–700 μg/mL, with an inhibition rate as high as 84%. Mass spectrometry analysis and sequence determination revealed a molecular weight of 685.378 Da and the sequence of Glu-Gln-Arg-Pro-Arg. This peptide has potential as a nutritional agent for anticancer treatment[33]. A new quinoa peptide was isolated from quinoa protease hydrolysate, and its effect on Caco-2 cell viability was determined. Based on histone deacetylase 1 (HDAC1) inhibitory activity assay results, peptides FHPFPR, NWFPLPR, and HYNPYFPG with high anticancer activity were screened. Molecular docking revealed that all three peptides bound to the active site of HDAC1[34]. After treatment of cancer cells with different walnut

protein hydrolysates, walnut protein alkaline protease hydrolysate (WPH-Al) was found to have the strongest cancer inhibitory effect. After WPH-Al treatment, HCT-116 cells became loosely arranged, exhibited lower density, and their colony-forming ability was significantly reduced. WPH-Al significantly inhibited the proliferation of HCT-116 cells in a dose-dependent manner, with the < 1 kDa fraction showing the strongest inhibitory effect, with an IC_{50} of 539.20 μg/mL. The three peptides with the highest relative abundance were Pro-Ile-Ser-Leu-Lys-Ser-Glu, Val-Ser-Leu-Pro, and Ser-His-Thr-Leu-Pro. Molecular docking revealed that these peptides had strong binding affinities for matrix metalloproteinase (MMP)-9 and caspase-3 and could form stable interactions with them[35].

After 8 weeks of gavage with quinoa protein or its hydrolysate in a mouse model of colorectal cancer induced by azoxymethane/dextran sulfate sodium (AOM/DSS), disease activity index (DAI) were reduced to varying degrees, colon shortening was significantly hindered, the number of polyps was significantly reduced, and the intestinal epithelium and crypts were significantly restored compared to the control group[36]. In nude mice transplanted with DLD-1 tumors, tumor volume and size were significantly reduced after FMBP treatment[31]. Glycine, a novel natural peptide isolated from pea seeds, reduced tumor volume by 38% in xenograft mice with CT-26 cells. These findings highlight the anticancer potential of glycine on colon cancer cells and suggest that it could be another promising inhibitory component of plant-derived peptides for colon cancer treatment[37]. Peptides extracted from non-digestible parts of common beans alleviated the disease severity of AOM/DSS-induced colon cancer in mice. Proteolytic peptide administration markedly declined the DAI and significantly decreased the number of tumors compared to the untreated group[38].

2.2 Alleviating effects of animal-derived protein-derived peptides on colon cancer

In Caco-2 cells treated with milk-derived bioactive peptide K-8-K (KVLPVPEK), downregulation of pro-inflammatory cytokines gene expression was observed, along with upregulation of the anti-inflammatory cytokine gene[39]. After treating human colon cancer cell line DLD-1 with SPP for 72 h, different concentrations of SPP significantly inhibited cell proliferation, and flow cytometry showed that SPP markedly increased DLD-1 cell apoptosis. SPP also had significant effects on mitochondrial metabolic processes and glycolytic pathways in DLD-1 cells, effectively reducing intracellular energy metabolism by inhibiting these key metabolic activities[20]. Casein and whey protein were purified from goat milk, and the casein protein hydrolysates (CPH) and whey protein hydrolysates (WPH) were obtained through hydrolysis with gastrointestinal peptidases. Treatment with CPH and WPH peptides markedly suppressed the production of tumor necrosis factor-α (TNF-α) and interleukin (IL)-6 in HT-29 cells[40]. Bovine lactoferricin (LfcinB), a peptide segment produced by the hydrolysis of bovine lactoferrin (Lf) by gastric protease, reduced the viability of HT-29 cells and significantly increased apoptosis, as shown by a LIVE/DEAD cell viability assay. However, this phenomenon did not occur in healthy human normal intestinal epithelial cells[41]. Ovotransferrin (OTF), one of the main components in egg white protein, exhibited the strongest cytotoxic activity with an IC_{50} of 0.92 mg/mL for HT-29 cells when hydrolyzed using a combination of promod 278P and thermolysin[42]. Camel whey protein hydrolysate (CWPH) was produced by hydrolysis at different temperatures, time points, and enzyme concentrations. CWPH showed increased anti-inflammatory effects, with values ranging from 732.50 to 3 779.16 μg diclofenac sodium equivalent (DSE)/mg prot. CWPH significantly inhibited the growth of HCT-116 cells[43]. Loach protein hydrolysates (LPH) hydrolyzed by papain were graded. These hydrolysates were classified as LPH-I (molecular weight > 10 kDa), LPH-II (molecular weight 5–10 kDa), LPH-III (molecular weight 3–5 kDa), and LPH-IV (molecular weight < 10 kDa). These fractions suppressed the growth of Caco-2 cells, with the LPH-IV fraction exhibiting the strongest antiproliferative effect, reducing Caco-2 colonocyte proliferation by approximately 6%, which was 12.8- and 8.7-fold lower than Caco-2 colonocytes treated with fractions LPH-I and LPH-II, respectively[44]. The skimmed yolk protein underwent hydrolysis using pepsin and trypsin, followed by purification through gel filtration, resulting in the anti-proliferative yolk gel filtration component (EYGF-33). The experimental results showed that when Caco-2 cells were treated with a dose of 1.0 mg/mL EYGF-33, significant inhibition of cell growth was observed after 48 h. In contrast, when HCEC cells were treated under the same conditions, no significant morphological changes were observed[45]. Peptides with antioxidant activity were isolated from crude peptide extracts of probiotic yogurt. Two *β*-casein-derived peptides were identified: ^{193}YQEPVLGPVRGPFPIIV^{209} and ^{69}SLPQNIPPLTQTPVVVVPPF^{87} (P17 and P19). Both peptides demonstrated notable inhibition rates of 41.49% and 38.55% against the HT-29 cells[46]. Similarly, treating the HCT-116 colon cancer cell line with camel Lf at a concentration of 5 mg/mL led to a significant 56% reduction in cell proliferation after 48 h[47]. A new peptide, ubiquitin, with a molecular weight of approximately 8.6 kDa, was identified by matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF-MS) from cow milk. Compared to untreated control cells, treatment of Caco-2 cells with ubiquitin resulted in a significant decrease in cell number[48]. Alkaline protease was used to hydrolyze melittin, and their anticancer activity was evaluated. MTT results showed that treatment with natural bee venom peptides and their enzymatic digests inhibited the growth of HCT-166 cells, with IC_{50} ranging from (101 ± 5.56) to (194.9 ± 7.636) μg/mL compared to untreated cells after 72 h of treatment. The viability of normal lung fibroblasts was largely unaffected under the same conditions[49].

The KT2 peptide, sourced from the white blood cell extract of *Crocodylus siamensis*, was investigated for its anticancer properties using a nude mouse model of HCT-116 cell transplantation, established through subcutaneous injection. The findings revealed that the KT2 peptide effectively reduced tumor volume and weight in colon cancer xenografts. Additional experiments indicated that the KT2 peptide enhanced the expression of apoptotic proteins in tumor tissues, suggesting its role in promoting the activation of the apoptotic pathway[50]. Rats were injected with AOM to induce the development of colon cancer. After bovine Lf peptide treatment, the incidence of colon cancer was significantly reduced by 83%[51]. After

feeding whey protein or casein to colon cancer rats, the results showed that the tumor incidence in rats fed a whey protein diet was 40% lower than that in rats fed a casein diet[52]. In a clinical trial, consuming 3 g/d of bovine Lf for 12 months significantly reduced the growth of adenomatous colorectal polyps in patients aged 63 years or younger[53].

2.3 Alleviating effects of seafood-derived protein-derived peptides on colon cancer

Using *Synechococcus* sp. VDW protein as a raw material, trypsin was used for hydrolysis, and different fractions of protein-derived peptides were purified by ultrafiltration. Among the fractions, the peptide with a molecular weight below 3 kDa exhibited strong cytotoxicity towards cancer cells, particularly the SW620 colon cancer cell line[54]. Three gastrointestinal peptidases, pepsin, trypsin, and chymotrypsin, were used to extract and hydrolyze the whole protein of *Spirulina platensis*. The hydrolysate was separated by gel filtration chromatography to obtain four components (Tr1–4). A novel peptide, HVLSRAPR, was identified from the Tr1 fraction and its effect on HT-29 cells was evaluated through cytotoxicity testing. The peptide exhibited a strong inhibitory effect on HT-29 cells, with an IC_{50} of 99.88 μg/mL[55]. Proteins extracted from *Dunaliella salina* were separated and hydrolyzed using trypsin and chymotrypsin. Peptide fractions and extracted proteins were used to assess their effects on SW-480 cells. After 24 h, a significant growth inhibitory effect was observed, particularly for the molecular weight 3 kDa peptide fraction. Further treatment at 72 h showed a significant enhancement of growth inhibition in SW-480 cells by the 3 kDa peptide fractions, with a 70% inhibition rate[56]. C-phycocyanin (the major protein of cyanobacteria *Spirulina*) was digested with gastric protease to obtain chromopeptides, and different fractions of chromopeptides (90 μmol/L) significantly reduced the viability of Caco-2 cells. Chromopeptides with high antioxidant activity exhibited strong cytotoxic effects, suggesting that these peptides can reduce cell viability by interfering with the specific free radical balance in cancer cells[57]. Enzymatic hydrolysis of oysters was performed using microbial proteases to prepare protein hydrolysates. MTT assay was used to assess the potential cytotoxicity of protein hydrolysates on two cell lines (Vero and HT-29). The experimental results showed that the protein hydrolysate did not exhibit significant cytotoxic effects on Vero cells. However, for the HT-29 cell line, the enzymatic hydrolysate exhibited strong cytotoxic effects, with IC_{50} of (90.31 ± 0.45), (70.87 ± 0.82), and (60.21 ± 0.45) μg/mL at 24, 48, and 72 h, respectively[58]. A novel anti-tumor peptide P6 (2 794.8 Da) was identified from a marine-based traditional Chinese medicine. This peptide, characterized by a sequence rich in lysine residues, significantly suppressed the proliferation of HT-29 and DLD-1 cells and effectively induced apoptosis. P6 also effectively induced cell apoptosis, with the apoptotic process accompanied by the formation of apoptotic vesicles, highlighting its potential in anti-tumor therapy[59]. The anticancer properties of Indian mackerel protein hydrolysate obtained using different enzymes and hydrolysis times were studied. The MTT assay results showed that the protein peptide hydrolyzed by alkaline protease for 30 min had the strongest inhibitory effect (78.71%) on SW-742 cells[21]. Rainbow trout fish skin was used as a raw material, hydrolyzed by alcalase and flavourzyme, and separated by ultrafiltration. These fractions were classified according to their molecular weights as < 3, 3–30, and > 30 kDa. Peptides with molecular weights less than 3 kDa exhibited the highest inhibition against HCT-116 cells in the MTT assay[60]. Low molecular weight peptides extracted from flathead by-products exhibited up to 91.04% growth inhibition against HT-29 cells[61]. Pepsinase was used to hydrolyze shrimp shell protein, producing fractions with molecular weights < 10, 10–30, and > 30 kDa. These fractions were evaluated for their inhibitory effects on colon cancer growth. Peptide fractions (< 10 and 10–30 kDa) exhibited significant inhibitory effects on the growth of Caco-2 cells, with an inhibition rate of 60%[62]. In the HT-29 cell nude mouse subcutaneous transplantation tumor model, compared with the control group, the tumor volume and weight of mice treated with P6 were significantly reduced. After treatment with 30 mg/kg P6, the inhibition rate of tumors reached 72.66%. Furthermore, after TUNEL staining treatment, the results showed that P6 induced significant apoptosis in tumor tissue cells[59].

2.4 Alleviating effects of microorganism-based protein-derived peptides on colon cancer

Bacteriocins are cationic peptides synthesized by ribosomes and secreted by almost all bacteria. Some bacteriocins have demonstrated selective cytotoxicity against cancer cells compared to normal cells, making them promising candidates for further anticancer research and clinical trials.

Colistin is a high molecular weight (> 20 kDa) antimicrobial peptide secreted by *Escherichia coli*. The *in vitro* antiproliferative effects of four colistins (A, E1, E3, U) on 11 human cancer cell lines were determined by MTT assay. The results showed that colistin had no significant effect on the growth of normal human fibroblast cell lines, while colistins A and E1 inhibited most of the cancer cell lines, with colistin A exhibiting the highest cytotoxicity against HT-29 cells[63]. A pediocin was isolated from *Pediococcus acidilactici* K2a2-3, with a molecular weight of 4.6 kDa. The inhibitory effect of bacteriocin fractions at concentrations of 800 and 1 600 AU/mL on the growth of HT-29 cells was determined by MTT assay. The results showed inhibition rates of (53.7 ± 7.0)% and (55.0 ± 4.8)% for the two bacteriocin concentrations, respectively[64]. Nisin is a polycyclic antimicrobial peptide with 34 amino acid residues, produced by *Lactococcus lactis*. A study reported the cytotoxicity of nisin against two types of colon cancer cells, HT-29 and Caco-2, with IC_{50} of 89.9 and 115 μmol/L, respectively[65]. Nisin was found to down-regulate the expression of metastasis-related genes such as *MMP-2* and *MMP-9*, cycle inhibitory factors (*Cif*), and cytolethal distending toxins (*CDTs*). It also reduced the secretion of carcinoembryonic antigens and up-regulated the tumor suppressor gene *LS-180* in HT-29, SW-480, and Caco-2 cell lines[66]. Microcin E492 is a low molecular weight (7 887 Da) bacteriocin secreted by *Klebsiella pneumoniae* RYC 492. Microcin E492 exhibited strong cytotoxic effects on HT-29 cells, inducing apoptosis. However, SW-620 cells showed greater resistance to the effects of microcin E492, although both cell types demonstrated significant sensitivity to its toxic effects. In *in vivo* experiments, intra-tumor injection of microcin E492 significantly reduced tumor size in a zebrafish SW-620 xenograft model. This study provides the first evidence of the *in vivo* antitumor properties of bacteriocins in an animal model and supports the potential of bacteriocins for developing novel anti-colon cancer therapies[67].

The alleviating effects of protein peptides from different sources on colon cancer are illustrated in Figure 1.

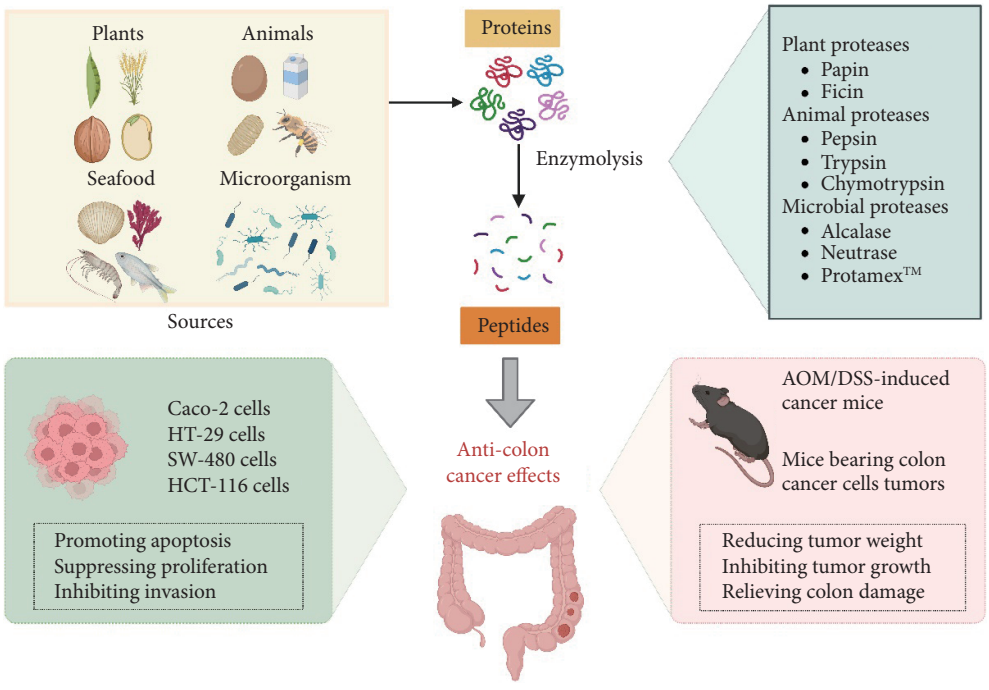

Figure 1 Alleviating effects of protein peptides from different sources on colon cancer (created in BioRender.com).

3 Mechanisms of protein-derived peptides in alleviating colon cancer

3.1 Inhibition of cancer cell proliferation and induction of apoptosis

Research has shown that the cell cycle of colon cancer cells changes after treatment with some protein-derived peptides. For example, cells in the G0–G1 phase treated with different hydrolysates of fenugreek protein, purafect-fenugreek proteins hydrolysate and esperase-fenugreek proteins hydrolysate, were 1.6 and 1.5 times higher than those in the control group, respectively[22]. Notable changes were observed in the cell cycle of HT-29 cells treated with HRBE, showing an increase in the proportion of cells in the G0/G1 phase and a reduction in those in the S and G2/M phases[29]. EYGF-33 treatment of Caco-2 cells resulted in a significant decrease in the population of cells in the G1 phase and a dramatic increase in the population of cells in the S phase[45]. Bovine Lf peptide treatment prolonged the S phase of the Caco-2 cell cycle, and the expression level of cyclin E1 was also reduced[68]. Several genes play key roles in maintaining normal cell cycle function. The expression levels of these genes also change significantly during cell cycle transitions. Specifically, the expression of cell cycle-related proteins such as cyclin-dependent kinase 2 (CDK2), cyclin D1, and cyclin B1 was significantly down-regulated, while the expression of cyclin-dependent kinase inhibitors p21 and p27 was upregulated[24–25,32,37,69]. In conclusion, protein-derived peptides possess significant inhibition on colon cancer cells. They effectively regulate the expression of cell cycle protein genes to block the normal progression of the cell cycle, thereby exerting anti-cancer effects at the cellular level.

Apoptosis-related features, such as apoptotic body formation, cytoplasmic contraction, membrane blebbing, and chromatin condensation were observed after treatment with protein-derived peptides, indicating that the cells underwent apoptosis[29,59]. Walnut protein peptide dose-dependently suppressed the growth of HCT-116 cells. The apoptosis rates of the 0.5 and 1 mg/mL WPH-Al treatment groups increased from (16.87 ± 0.82)% in the control group to (36.80 ± 4.92)% and (41.50 ± 5.28)%, respectively[35]. HRBE induced apoptosis in a dose-dependent manner. After treating SW-620 cells with 1.25, 2.5, 5, and 10 mg/mL HRBE for 72 h, the percentage of apoptosis was 16%, 51%, 60%, and 76%, respectively[29]. HCT-116 and HT-29 cells treated with BG-4 at a concentration of 125 μg/mL showed a significant increase in apoptosis after 16 h of treatment. Specifically, in the control group, without any intervention, the apoptosis rates of these two cancer cells were only 5.4% and 5.8%, respectively. However, after BG-4 treatment, their apoptosis rates increased to 24.8% and 31.9%, respectively[28]. Lunasin treatment doubled the percentage of apoptotic cells in comparison to the control cells[25]. Additionally, protein-derived peptides can promote apoptosis by decreasing mitochondrial membrane potential and increasing reactive oxygen species (ROS) levels. P6 not only reduced the mitochondrial membrane potential but also affected intracellular ion concentrations and ROS levels. P6 increased the intracellular Ca^{2+} content and ROS levels[59]. EYGF-33 significantly enhanced ROS production in Caco-2 cell mitochondria[45]. During apoptosis, the levels of several genes and proteins change, with cleaved poly(ADP-ribose) polymerase (PARP) and cleaved caspase-3 showing increased expression levels. Additionally, studies revealed an upregulation of the pro-apoptotic factor Bax, further promoting the apoptotic process, while the expression of the anti-apoptotic factor Bcl-2 was suppressed[32,37,50,59]. The F_A (molecular weight < 3 kDa) fraction of *Synechococcus* sp. VDW protein hydrolysates triggered the apoptotic pathway in SW620 cells after 24, 48, and 72 h of treatment, with the most significant activation of caspase-3, caspase-8, and caspase-9 occurring at the 72-h mark[54]. P6 significantly enhanced p38 phosphorylation in two colorectal cancer cell lines, suggesting that P6-induced mitochondrial apoptosis in colorectal cancer cells is partially mediated by activating the p38-mediated mitogen-activated protein kinase (MAPK) signaling pathway[59]. Both LfcinB and bovine Lf increased the expression of key proteins involved in tumor suppression, such as caspase-8, p53, and p21[41]. Significant changes were observed in

lunasin-treated cells compared to controls. Specifically, the Bcl-2 to Bax ratio decreased from 8.5 to 0.4, suggesting that the cells entered a more active apoptotic process. The activity of caspase-3 was significantly enhanced by 77%, while the expression of pro-apoptotic nuclear cluster proteins, essential for initiating and accelerating apoptosis, surged more than fivefold[25]. In tumor tissues of xenograft mice, bioactive peptide treatment elevated the *PARP* and *p53* expression, while decreasing the expression of myeloid cell leukemia 1 (*Mcl-1*). This indicated that the active peptide effectively inhibited the growth of human colorectal tumor cells and induced apoptosis by activating the PARP-p53-Mcl-1 signaling pathway[70].

In summary, protein-derived peptides inhibit cell growth by regulating cyclin genes to block the cell cycle. They also regulate the expression of genes and proteins involved in apoptosis, thereby inhibiting colon cancer development and alleviating its severity.

3.2 Enhancement of antioxidant activity

The antioxidant activity of enzymatic hydrolysates is influenced by the type of enzyme and the concentration of the hydrolysates, leading to significant differences in antioxidant activity among different hydrolysates. Experiments were conducted to study the antioxidant activity of different enzymes on CPP hydrolysate. The hydrolysates exhibited trolox-equivalent antioxidant capacities ranging from 0.23 to 2.75 mmol/L. Additionally, the protein hydrolysates exhibited 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and hydroxyl radical scavenging activities ranging from 33.3% to 64.8% and 33.7% to 63.2%, respectively. Further, the protein hydrolysates showed high affinity for metal ion chelation, with iron and copper chelation capacities of 33.2%–62.5% and 30.2%–50.5%, respectively. In terms of total antioxidant activity, the hydrolysates exhibited very high antioxidant activity, ranging from 0.65 to 0.85, with the highest antioxidant activity observed in the pepsin-hydrolyzed product[27]. Among the protein-derived peptides of *Synechococcus* sp. VDW, the F_A fraction exhibited the strongest scavenging activity against 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) cation radical and DPPH radical. Four sub-fractions (F1–4) were obtained by separating the F_A fraction using reverse-phase high performance liquid chromatography (HPLC). The F4 subcomponent exhibited the highest ABTS cation radical scavenging activity and was selected for electrospray ionization quadrupole TOF-MS (ESI-Q-TOF-MS) analysis. The peptide AILESYSAGKTK exhibiting the strongest ABTS cation radical scavenging activity was identified[54]. Oyster protein hydrolysate was purified, resulting in a protein fraction with strong antioxidant activity. Further purification of the active peptide components using ultra-high performance liquid chromatography-MS yielded a total of seven antioxidant peptides. Among the seven peptides (SCAP1–7), three peptides (SCAP1, SCAP3, and SCAP7) exhibited the strongest DPPH radical scavenging activity. The amino acid sequences of these three peptides, analyzed by ESI-Q-TOF-MS, were LANAK, PSLVGRPPVGKLTL, and VKVLLEHPVL[58]. Under different enzyme treatments and treatment times, the mackerel hydrolyzed protein peptide produced by alkaline protease hydrolysis for 30 min exhibited the strongest DPPH radical scavenging activity (90.42%), highest iron reduction capacity (0.37 μmol/g), and highest ABTS cation radical scavenging activity (91.00%)[21]. Enzymatic hydrolysis of rainbow trout skin was carried out using Flavourzyme and Alcalase enzymes. The results showed that the peptides produced after both enzymatic treatments exhibited antioxidant and anticancer activities. Specifically, the flavourzyme-hydrolyzed product was more effective than the alcalase-hydrolyzed product in the DPPH radical scavenging and iron reduction reaction[60]. The two-step enzymatic hydrolysate (promod 278P + thermolysin) of OTF exhibited strong antioxidant activity and stronger anti-cancer activity compared to the single-step enzymatic hydrolysate and OTF. The OTF protease hydrolysate also exhibited significant DPPH radical, NO, and ABTS cation radical scavenging activity[42]. Three proteolytic enzymes were employed to hydrolyze walnut kernel protein to extract peptides. Among them, the peptide produced by chymotrypsin hydrolysis displayed the highest antioxidant and anticancer activity. In antioxidant assays, the obtained peptides exhibited ABTS cation radical scavenging activity and ROS inhibition. Additionally, the findings suggested a significant correlation between the antioxidant and anticancer activities[26]. The low molecular weight peptide (3 kDa) significantly inhibited radicals and exhibited the highest radical scavenging activity on DPPH radical and ABTS cation radical, with scavenging rates of 94.03% and 82.89%, respectively. Further purification resulted in a new peptide with radical scavenging activity properties: MGPGLAGAPGEAGR[61]. The hydrolysate of loach protein hydrolyzed by papain exhibited the strongest oxygen radical absorbance capacity (ORAC) (reaching (215 ± 5.9) mmol trolox/100 g loach peptide when the concentration was 60 μg/mL)[44]. Antioxidant peptides P17 and P19 were purified from yogurt. Among them, P17 showed high scavenging activity against ABTS cation radical, with an IC_{50} of 29.88 μg/mL, while P19 had an IC_{50} of 1.44 mg/mL[46]. Chromopeptides obtained from the digestion of C-phycocyanin were separated by HPLC and analyzed for their potential biological activity. The study showed that all five chromopeptides components exhibited significant antioxidant properties and metal ion-chelating ability. Furthermore, these chromopeptides prevented radical-induced hemolysis of human erythrocytes by relying on their antioxidant properties. In terms of cytotoxic activity against Caco-2 cells, the performance of chromopeptides showed a high correlation with ORAC (r = 0.93), reducing capacity (r = 0.88), and copper ion-chelating capacity (r = 0.93), implying that chromopeptides with higher antioxidant activity have higher cytotoxicity[57]. While antioxidant peptides have demonstrated radical scavenging activity in numerous *in vitro* studies, additional research is necessary to assess their side effects, safety, and potential allergenicity for therapeutic applications. Additionally, *in vivo* studies are required to determine how these antioxidant peptides exert therapeutic effects on cancer.

3.3 Other mechanisms

Research has discovered that peptides found in the NDF of beans inhibit the proliferation of HCT-116 cells by interfering with the RAS and decreasing the activity of angiotensin II (Ang II)[71]. Some reports suggest that synthetic RAS inhibitors can reduce the incidence of colitis-associated colorectal cancer[72]. The activity of RAS depends on Ang II concentration, regulated by angiotensin-converting enzyme (ACE), angiotensinogenase, and their respective inhibitors[73]. Ang II type 1 receptor (AT1R) exists in colorectal tumors and is activated through different pathways, leading to Ang II production, which then stimulates cell proliferation and neovascularization, promoting tumor growth[74]. Protein-derived peptides can alleviate colorectal cancer by inhibiting the RAS. Peptides produced by hydrolyzing casein and whey protein significantly inhibited ACE activity[40]. These peptides can also reduce the levels of TNF-α and IL-6 in HT-29 cells. When Caco-2

cells were treated with bioactive peptides K-8-K and S-10-S derived from milk and soybeans and stimulated by TNF-α, the gene expression of *IL-1β* and *IL-6* was downregulated, while the expression of the anti-inflammatory cytokine gene was upregulated[39]. In lipopolysaccharide-induced RAW264.7 macrophages, treatment with soy protein-derived peptides inhibited the production of NO and prostaglandin D_2[19]. This indicates that protein-derived peptides have anti-inflammatory effects. MMP-9 is a target of bioactive substances that allows cancer cells to metastasize by degrading the extracellular matrix[75]. Protein-derived peptides from mung beans, chickpeas, cowpeas, beans, and fava beans have been found to significantly reduce MMP-9 activity. Among them, peptides from lupin and lentil suppressed the growth of HT-29 cells, and peptides from lupin and chickpea significantly reduced cell migration[76]. Protein-derived peptides have the potential to inhibit cancer cell metastasis. Among the anti-cancer peptides isolated from walnut protein, MMP-9 was identified as a candidate core target, and molecular docking was performed. The results indicated that the peptide had desirable binding affinity and stable interaction with MMP-9[35]. Protein-derived peptides can target MMP-9 and suppress its activity to decrease colon cancer cell metastasis.

The mechanisms of protein-derived peptides in alleviating colon cancer are shown in Figure 2.

This review summarizes research on the inhibitory effects of protein-derived peptides against colon cancer. Cellular experiments are detailed in Table 1 and animal experiments in Table 2.

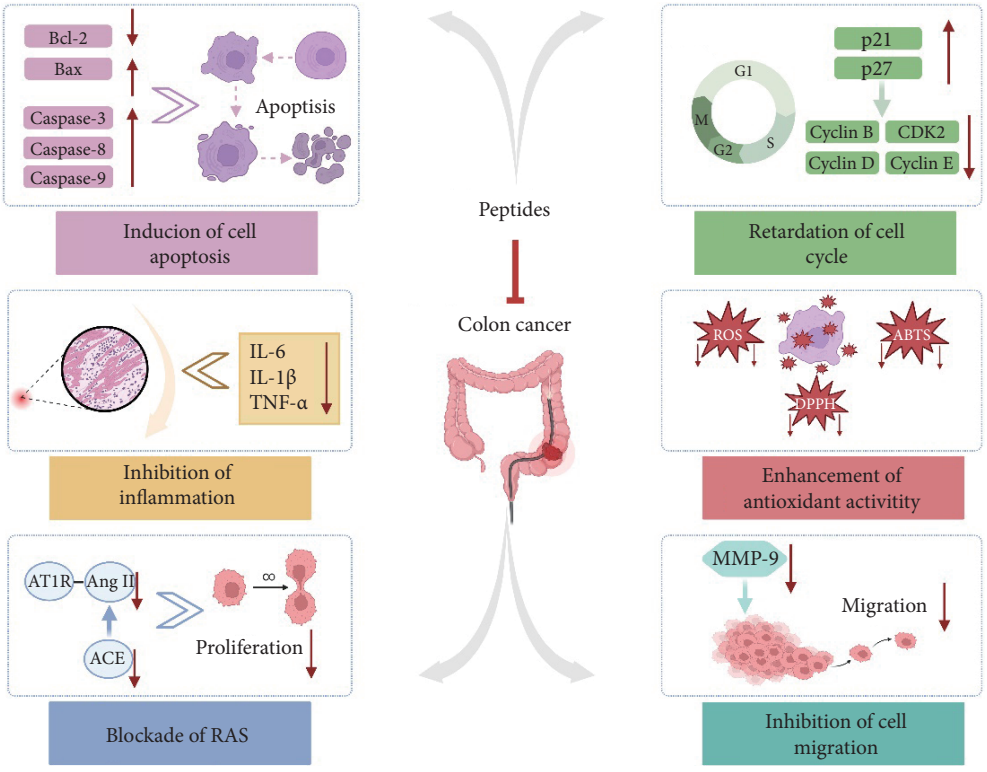

Figure 2 Mechanisms of protein peptides in alleviating colon cancer (created in BioRender.com).

Table 1 *In vitro* cellular studies on the inhibitory effects of protein-derived peptides on colon cancer.

Sources of peptide	Model	Molecular mass	Amino acid composition	Results	Reference
Fenugreek	Caco-2/TC7 cells	No available results	Hydrophobic, aromatic, and positively charged amino acids	Reduced cell viability, enhanced intrinsic apoptosis, G1 phase obstruction	[22]
Sprouted soybeans	Caco-2, HT-29, and HCT-116 cells	>10, 5–10, and < 5 kDa	No available results	Peptides with a higher molecular weight are more effective in inhibiting cell proliferation compared to those with a lower molecular weight	[11]
Pea seed	CT-26, SW480, and NCL-H716 cells	No available results	Vglycin	Inhibition of cell growth, vitality, and colony formation, downregulated expression of proliferating cell nuclear antigen, promotion of apoptosis, cell cycle arrest in G1/S phase	[37]
Walnut	HCT-116 cells	< 1 kDa	PISLKSE, VSLP, and SHTLP	Reduced the ability of cells to form colonies, reduced colony formation, peptides hydrolyzed by alkaline proteases into smaller molecules exhibit the strongest inhibitory effect, promotion of apoptosis	[35]
Bean	HCT-116, RKO, and KM12L4 cells	< 1 kDa	GLTSK, LSGNK, GEGSGA, MPACGSS, and MTEEY	Reduced cell proliferation, induction of cell cycle arrest and mitochondrial apoptosis pathways	[24]

Table 1 (*Continued*)

Sources of peptide	Model	Molecular mass	Amino acid composition	Results	Reference
Corn pollen	HT-29 cells	10–72 kDa	Essential, antioxidant, hydrophobic, and hydrophilic amino acids	Reduced cell viability	[27]
Synechococcus sp. VDW	SW-620 cells	< 3 kDa	AILESYSAGKTK	Activation of apoptotic pathways, enhanced activity of caspase-3, caspase-8, and caspase-9	[54]
Oysters	HT-29 cells	< 2 kDa	Leu-Ala-Asn-Ala-Lys, Pro-Ser-Leu-Val-Gly-Arg-Pro-Pro-Val-Gly-Lys-Leu-Thr-Leu, and Val-Lys-Val-Leu-Leu-Glu-His-Pro-Val-Leu	Significant cytotoxic effect	[58]
Riceberry rice bran	HT-29 and SW-620 cells	> 50, 50–30, 30–10, 10–3, and < 3 kDa	No available results	Greater toxic effect on SW-620 cells compared to HT-29 cells, promotion of cellular aging and apoptosis in SW-620 cells, cell cycle arrest in HT-29 cells	[29]
Marine Chinese medicine	HT-29, HCT-116, SW-620, and DLD-1 cells	2 794.8 Da	WYIRKIRRFFKWLKKKLKKK	Inhibition of proliferation and colony formation in HT-29 and DLD-1 cells, induction of apoptosis, cell cycle arrest in the S phase, activation of the mitochondrial apoptosis pathway via MAPK signaling pathway	[59]
Indian mackerel	SW-742 cells	No available results	Lysine, arginine, glutamic acid, and aspartic	Protein hydrolyzed by alkaline protease for 30 min shows the strongest inhibitory effect	[13]
Silkworm chrysalis	DLD-1 cells	No available results	No available results	Inhibition of cell proliferation, promotion of apoptosis, inhibition of mitochondrial metabolism and glycolysis, reduction in cellular energy metabolism	[12]
Casein and whey protein in goat milk	HT-29 cells	25–75 kDa	No available results	Inhibition of the production of pro-inflammatory cytokines	[40]
Bovine Lf	HT-29 cells	No available results	No available results	Reduced cell viability, increased expression of key tumor suppressor proteins (caspase-8, p53, and p21)	[41]
High oleic acid soybean	HCT-116 cells	< 5, 5–10, and 10–50 kDa	No available results	Inhibition of cell growth	[23]
Rainbow trout skin	HCT-116 cells	< 3, 3–30, and > 30 kDa	No available results	Peptides with a molecular weight less than 3 kDa exhibit the highest inhibitory effect	[60]
OTF	HT-29 cells	<40 kDa	No available results	Hydrolysate prepared by combining two enzymes shows the strongest cytotoxic effect	[42]
Walnut	HT-29 cells	No available results	No available results	Inhibition of cell vitality	[26]
Flathead by-products	HT-29 cells	<3 kDa	Met-Gly-Pro-Pro-Gly-Leu-Ala-Gly-Ala-Pro-Gly-Glu-Ala-Gly-Arg	Inhibition of cell growth	[61]
Camel whey protein	HCT-116 cells	No available results	AHLEQVLLR and ALPNIDPPTVER	Inhibition of cell growth, induction of cell cycle arrest in G2/M phase	[43]
Rice bran	Caco-2 and HCT-116 cells	685.378 Da	Glu-Gln-Arg-Pro-Arg	Inhibition of cell proliferation	[33]
Spirulina platensis	HT-29 cells	935.1 Da	HVLSRAPR	Inhibition of cell proliferation	[55]
Dunaliella salina	SW-480 cells	<3, 3–10, and >10 kDa	No available results	Peptide fraction with a small molecular weight exhibits the strongest inhibitory effect on cell growth	[56]
Loach	Caco-2 cells	< 3, 3–5, 5–10, and > 10 kDa	No available results	Inhibition of cell proliferation	[44]
Momordica charantia	HCT-116 and HT-29 cells	4 kDa	No available results	Inhibition of cell proliferation, promotion of apoptosis, increased expression of Bax and caspase-3, decreased expression of Bcl-2	[28]
Sweet potato	HT-29 cells	> 10, 5–10, 3–5, and <3 kDa	No available results	Small molecular weight peptides exhibit the strongest antiproliferative activity, cell cycle arrest in G2/M phase, increased expression of p21, induction of apoptosis via reduced Bcl-2 expression, increased Bax expression, and activated caspase-3, inhibition of cell migration	[32]
Soybean lunasin	HT-29 cells	No available results	Arginine-glycine-aspartic acid motif	Stimulation of apoptosis via activation of the apoptotic mitochondrial pathway and induction of pro-apoptotic nuclear proteins	[25]

Table 1 (*Continued*)

Sources of peptide	Model	Molecular mass	Amino acid composition	Results	Reference
Soybean lunasin	KM12L4 cells	No available results	Arginine-glycine-aspartic acid motif	Cell cycle arrest in G2/M phase, increased expression of cyclin-dependent kinase inhibitors p21 and p27, activation of apoptotic mitochondrial pathway	[69]
Yolk	Caco-2 cells	No available results	No available results	Inhibition of cell proliferation, cell cycle arrest in S phase	[45]
Yogurt	HT-29 cells	< 3 kDa	YQEPVLGPVRGPFPIIV and SLPQNIPPLTQTPVVVPPF	Inhibition of cell proliferation, cell cycle arrest in G2/M phase, promotion of apoptosis	[46]
Foxtail millet bran	DLD1, SW-480, and HT-29 cells	35 kDa	No available results	Inhibition of cell growth, induction of G1 phase blockade, induction of apoptosis, induction of mitochondrial membrane depolarization	[31]
C-Phycocyanin	Caco-2 cells	No available results	No available results	Reduction of cell viability by disrupting the free radical balance specific to cancer cells	[57]
Camel Lf	HCT-116 cells	No available results	No available results	Inhibition of cell proliferation, inhibition of DNA damage	[47]
Melittin	HCT-116 cells	28–36 kDa	GIGAVLKVLTTGLPALISWIKRKRQQ	Inhibition of cell growth	[49]
Shrimp shell	Caco-2 cells	< 10, 10–30, and > 30 kDa	No available results	Low molecular weight peptide fractions exhibit a stronger inhibitory effect on cells	[62]
Escherichia coli	HT-29 cells	20 kDa	No available results	Inhibition of cell growth	[63]
Pediococcus acidilactici K2a2-3	HT-29 cells	4.6 kDa	No available results	Inhibition of cell proliferation	[64]
Lactococcus lactis	HT-29, SW-480, and Caco-2 cells	3.5 kDa	No available results	Down-regulation of the expression of genes associated with metastasis, up-regulation of tumor suppressor genes, reduction of carcinoembryonic antigen secretion	[66]
Klebsiella pneumoniae RYC 492	SW-620 and HT-29 cells	7 887 Da	No available results	Inhibition of cell growth	[67]

Table 2 *In vivo* animal studies on the inhibitory effects of protein-derived peptides on colon cancer.

Sources of peptide	Model	Molecular mass	Amino acid composition	Results	Reference
Quinoa	AOM/DSS-induced colon cancer mouse model	No available results	No available results	Reduced DAI score, prevented colon shortening, significant reduction in the number of polyps, recovery of intestinal epithelium and colonic crypts, increased short chain fatty acids content in colon tissue, reduced pathogenic bacteria, increased probiotics	[36]
Marine Chinese medicine	HT-29 cell subcutaneous transplanted tumor model in mice	2 794.8 Da	WYIRKIRRFFKWLKKKLKKK	Decreased tumor volume and weight, improved tumor suppression rate, induced apoptosis in tumor tissue cells	[59]
White blood cell extract of *Crocodylus siamensis*	HCT-116 cell transplanted tumor model in mice	No available results	No available results	Reduced tumor volume and weight, increased expression of pro-apoptotic factors, decreased expression of anti-apoptotic factors	[50]
Foxtail millet bran	DLD-1 cell transplanted tumor model in mice	35 kDa	No available results	Significant reduction in tumor volume and size, tumors exhibited significant apoptosis and growth inhibition	[31]
Pea seed	CT-26 cell transplanted tumor model in mice	No available results	Vglycin	Tumor volume reduction	[37]
Common bean	AOM/DSS-induced colon cancer mouse model	No available results	No available results	Decreased DAI, reduced the number of tumors	[38]
K. pneumoniae RYC 492	SW-620 cell transplanted tumor model in zebrafish	7 887 Da	No available results	Significant reduction in tumor size	[67]

4 Factors affecting the anti-cancer activity of protein-derived peptides

Obtaining bioactive peptides from food proteins is currently the most widely used method. Specific proteolytic enzymes are used to hydrolyze proteins, releasing many peptide fragments in the hydrolysis products. These products are then classified and purified based on their structural characteristics. The potential health benefits of these fractions are tested *in vitro*. Peptides are further isolated from the fractions that showed the highest capacity in the *in vitro* activity assay and identified using LC-MS[77]. The amino acid composition, chain length, and sequence of proteins determine the presence of peptides with the desired biological activity[78–79]. The biological activity of protein-derived peptides depends on several factors, including protein source, enzyme type, pretreatment (ultrasound, microwave, pulsed electric field), operating conditions (time, temperature, pH), hydrolysis degree, peptide structure (amino acid composition, sequence, molecular weight, surface and molecular hydrophobicity, net charge, and spatial conformation), etc.[80–81]. In studying the inhibitory effects of protein-derived peptides on colon cancer, it has been found that peptides obtained from different sources have different inhibitory abilities on colon cancer cells. For example, the anti-cancer effects of protein-derived peptides obtained from the same treatment of different soybean strains vary[23]. In general, peptides with smaller molecular weights possess greater molecular mobility and permeability compared to those with larger molecular weights, leading to enhanced anti-tumor effects[60,82]. Depending on the enzyme type and species used, the obtained peptide sequences vary, as do the biological functions performed by the peptide. Different processing times using different enzymes yield varying results. For example, the characteristics of mackerel hydrolyzed protein products obtained at different hydrolysis times (10, 20, and 30 min) using alkaline protease and flavor protease were studied. The results showed that alkaline protease hydrolysis for 30 min had the highest degree of hydrolysis and the strongest inhibitory effect on SW-742 cells[21]. The type and composition of amino acid residues affect the anticancer activity of peptides, particularly hydrophobic positively charged peptides (such as lysine and glycine), which enhance cytotoxicity against cancer cells. Additionally, the secondary structure of peptides can promote peptide-cancer cell membrane interactions, contributing to cancer cell destruction and death[83–84].

The safety of bioactive peptides derived from food proteins is also an important factor limiting their activity. For example, during protein or peptide processing, biogenic amines, *D*-amino acids, lysinoalanine, and some allergens are formed[85]. Although no toxicity or adverse effects have been detected in current animal and human trials, there is still a lack of information on high-dose or long-term studies of peptides in population trials[86–87]. The relatively low bioavailability in the human body can lead to differences in the biological activity of protein-derived peptides *in vivo* and *in vitro*. The prerequisite for protein-derived peptides to exert beneficial effects in the body is resistance to degradation by gastrointestinal proteases and serum peptidases[80,88–89]. This stability ensures that peptide sequences exhibiting *in vitro* biological activity can safely reach the desired cellular site of action. Some marine-based peptides are limited in their applications due to their extremely low water solubility, chemical instability, and adverse gastrointestinal properties[90]. Additionally, bioactive peptides have multiple molecular targets, but it is unclear whether certain molecular targets may lead to potential side effects. In the context of anti-cancer applications, analyzing and exploring factors that affect the biological characteristics of protein-derived peptides is beneficial for revealing their molecular mechanisms and interactions. When developing functional peptide foods for anti-cancer purposes, these limitations should be addressed. Factors affecting the activity of protein-derived peptides are shown in Figure 3.

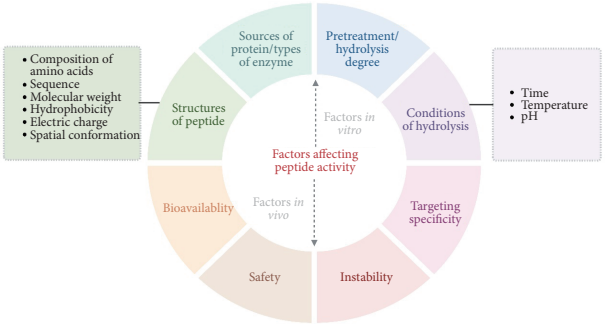

Figure 3 Factors affecting the activity of protein peptides (created in BioRender.com).

5 Conclusions

The causes of colon cancer are complex and varied, and current treatments for the disease have certain limitations and side effects. Extensive research and scientific evidence suggest that protein-derived peptides play a significant role in the prevention and treatment of colon cancer. This review summarizes the mitigating effects of bioactive peptides from plant, animal, seafood, and microbial sources on colon cancer, as well as the mechanisms through which these peptides exert their effects, primarily including the promotion of apoptosis and the enhancement of antioxidant activity. Additionally, this review briefly discusses the factors that influence the biological activity of these peptides.

Natural peptides offer several key benefits, such as a strong safety profile with minimal toxicity, a broad spectrum of therapeutic effects, fewer side effects compared to synthetic drugs, and enhanced absorption efficiency in the gastrointestinal tract. The easy availability and low cost of protein raw materials make the development of bioactive peptides from food proteins highly feasible. Furthermore, increased consumer awareness of functional foods and their health benefits has prompted researchers to explore the use of food-derived bioactive peptides in disease prevention and treatment. Advances in proteomics, bioinformatics, peptide libraries, and modification technologies will accelerate the development and use of bioactive peptides as innovative drugs in future clinical applications.

However, the application of bioactive peptides also presents challenges, such as potential intestinal wall damage, toxicity to erythrocytes and lymphocytes, radical generation, enzymopathological and immunopathological tissue damage, and cytotoxicity. The ingestion of these peptides may lead to various health complications. Although increasing evidences suggest that protein-derived peptides may have anticancer properties, further in-depth studies of their cell signaling pathways and *in vivo* experiments are required to validate their safety as effective treatments for certain cancers. Additional research into the beneficial effects of bioactive peptides on colon cancer, along with more clinical trials in humans, is essential to confirm their potential in colon cancer treatment.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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