A Narrative Review on Therapeutic Potential of Naringenin in Colorectal Cancer: Focusing on molecular and biochemical processes

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Abstract

Colorectal cancer (CRC) is a common and highly metastatic cancer affecting people worldwide. Drug resistance and unwanted side effects are some of the limitations of current treatments for CRC. Naringenin (NAR) is a naturally occurring compound found in abundance in various citrus fruits such as oranges, grapefruits, and tomatoes. It possesses a diverse range of pharmacological and biological properties that are beneficial for human health. Numerous studies have highlighted its antioxidant, anti-cancer, and anti-inflammatory activities, making it a subject of interest in scientific research. This review provides a comprehensive overview of the effects of NAR on CRC. The study’s findings indicated that NAR: 1) interacts with estrogen receptors, 2) regulates the expression of genes related to the p53 signaling pathway, 3) promotes apoptosis by increasing the expression of proapoptotic genes (Bax, caspase9, and p53) and downregulation of the antiapoptotic gene Bcl2, 4) inhibits the activity of enzymes involved in cell survival and proliferation, 5) decreases cyclin D1 levels, 6) reduces the expression of cyclin-dependent kinases (Cdk4, Cdk6, Cdk7) and anti-apoptotic genes (Bcl2, x-IAP, c-IAP-2) in CRC cells. In vitro CDK2 binding assay was also performed, showing that the NAR derivatives had better inhibitory activities on CDK2 than NAR. Based on the findings of this study, NAR is a potential therapeutic agent for CRC. Additional pharmacology and pharmacokinetics studies are required to fully elucidate the mechanisms of action of NAR and establish the most suitable dose for subsequent clinical investigations.
on molecular and biochemical processes

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Abstract

Colorectal cancer (CRC) is a common and highly metastatic cancer affecting people worldwide. Drug resistance and unwanted side effects are some of the limitations of current treatments for CRC. Naringenin (NAR) is a naturally occurring compound found in abundance in various citrus fruits such as oranges, grapefruits, and tomatoes. It possesses a diverse range of pharmacological and biological properties that are beneficial for human health. Numerous studies have highlighted its antioxidant, anti-cancer, and anti-inflammatory activities, making it a subject of interest in scientific research. This review provides a comprehensive overview of the effects of NAR on CRC. The study’s findings indicated that NAR: 1) interacts with estrogen receptors, 2) regulates the expression of genes related to the p53 signaling pathway, 3) promotes apoptosis by increasing the expression of proapoptotic genes (Bax, caspase9, and p53) and downregulation of the antiapoptotic gene...
Bcl2, 4) inhibits the activity of enzymes involved in cell survival and proliferation, 5) decreases cyclin D1 levels, 6) reduces the expression of cyclin-dependent kinases (Cdk4, Cdk6, Cdk7) and anti-apoptotic genes (Bcl2, x-IAP, c-IAP-2) in CRC cells. In vitro CDK2 binding assay was also performed, showing that the NAR derivatives had better inhibitory activities on CDK2 than NAR. Based on the findings of this study, NAR is a potential therapeutic agent for CRC. Additional pharmacology and pharmacokinetics studies are required to fully elucidate the mechanisms of action of NAR and establish the most suitable dose for subsequent clinical investigations.

**Keywords:** Naringenin, Colorectal cancer, Colon cancer, Apoptosis, Cell cycle arrest, Flavanone

**Significance statement:**

The review discusses the molecular and biochemical processes related to NAR’s effects on CRC, emphasizing its diverse pharmacological and biological properties.

NAR interacts with estrogen receptors, regulates gene expression related to the p53 signaling pathway, promotes apoptosis, inhibits enzymes involved in cell survival and proliferation, decreases cyclin D1 levels, and reduces the expression of cyclin-dependent kinases and anti-apoptotic genes in CRC cells.

*In vitro* studies demonstrate that NAR derivatives have better inhibitory activities on CDK2 than NAR, suggesting their potential as therapeutic agents for CRC.

**Abbreviations**

CRC: Colorectal cancer

NAR: Naringenin

CDK: Cyclin-dependent kinases

FAP: Familial adenomatous polyposis

ER: Estrogen receptors

COX: Cyclooxygenase

MAPK: Mitogen-activated protein kinases

MNDs: Modified Naringenin derivatives

ROS: Reactive oxygen species

LD50: Lethal dose 50%

5-FU: 5-fluorouracil

SEDDS: Self-emulsifying Drug Delivery Systems

DRIs: Dietary reference intakes

RDAs: Recommended daily allowances

FEN: Fermentation Extract of Naringenin

BPA: Bisphenol A

DS: Diosmin

ATF3: Activating transcription Factor 3

CREB: cAMP response element-binding protein

CDK2: Cyclin-dependent kinase 2

HMACF: High multiplicity aberrant crypt foci
AOM: Azoxymethane
Rb: Retinoblastoma protein
6CEPN: 6-C-(E-phenylethenyl)-Naringenin

Introduction

Colorectal cancer (CRC) as the third most prevalent neoplasm is characterized by the growth of abnormal cells inside the cellular lining of the intestinal and rectal tissue and eventually turn into tumors over time. The cancerous growth initiate in the inner colon lining and invade other colon layers or neighboring lymph nodes if left untreated (1-4). CRC predominantly affects the elderly, with most incidences found in individuals aged 50 and above. However, according to recent statistics, CRC has been increasing in the younger generation in many countries including the United States and Europe (5-7). By 2019, CRC was projected to be responsible for around 0.69 million mortalities, and its incidence was expected to rise by 1.5% annually among individuals under the age of 50 (8). In the year 2020, approximately 1.9 million newly diagnosed cases of CRC were identified worldwide, and this number will increase to 2.5 million by 2040 (1, 9). The likelihood of CRC escalates with advancing age and a positive family history of the condition. Certain genetically linked conditions, such as hereditary nonpolyposis colorectal cancer (HNPCC) or familial colorectal adenomas rise the likelihood of the disease (10). Individuals who have previously had colorectal polyps are more likely to develop new polyps or cancer in the future (11). CRC can be associated with a risk of recurrence and resistance to treatment, which can lead to metastasis. So, early detection and treatment of CRC may be important in preventing metastasis and improving outcomes (12, 13). Advanced or metastatic CRC usually has a low survival rate, with less than 10% of patients surviving. The main treatment approaches for this stage of CRC are radiotherapy and chemotherapy. A variety of interventions are accessible to manage or eliminate tumors, including radiotherapy, surgical resection, and chemotherapy, but they are linked with notable drawbacks and adverse reactions (14). Chemotherapy is one of the procedures to treat or remove tumors in CRC. It can be used before or after surgery, or as the primary treatment for advanced or metastatic CRC (15, 16). The chemotherapy drugs employed for CRC may differ based on the stage, site of the cancer, and individual factors like age and general well-being (17, 18). Standard chemotherapeutics used to treat CRC include the antimetabolite 5-fluorouracil (5-FU), as well as the targeted agents capecitabine, oxaliplatin, irinotecan, and the anti-angiogenic antibody bevacizumab (19, 20). In addition to being used in conjunction with additional modalities such as surgical intervention and radiotherapy, it can also be an effective treatment option for CRC (21, 22). Chemotherapy often entails notable adverse effects, including nausea, emesis, lethargy, alopecia, and increased risk of infection (23, 24). Phytochemicals are active compounds present in plants that have been demonstrated to offer numerous health benefits, including anticancer properties (25, 26). Phytochemicals prevent CRC through various processes, including antioxidative capacity, anti-inflammatory effects, and alteration of cellular signaling pathways, and regulation of cell proliferation and differentiation (27, 28). Some examples of phytochemicals that have been researched for their potential anticancer effects in CRC include flavonoids, carotenoids, phenolic acids, and glucosinolates (27, 29, 30). The numerous pharmacologic attributes of flavonoids including anti-oxidant, anti-mutagenic, anti-angiogenic, anti-inflammatory, and anti-cancer activity have made them a subject of interest. Therefore, flavonoids have been considered as promising agents for preventing cancer (31-35).

Flavonoids, particularly flavanones such as Naringenin (NAR, 4',5,7-trihydroxyflavonone), have been acknowledged for their ability to act as antioxidants and reduce inflammation in CRC. These compounds exert their effects through various mechanisms including activating estrogen receptors (ER) and inhibiting cell proliferation (36-38). NAR derivatives are created by modifying the structure of NAR, typically by adding or substituting different functional groups at specific positions on the molecule (39). The bulky substituents such as thiophencarboxylate, methylbenzoate, isobutyrate, allyloxy, and phenyl carbonate are attached to position 7 of the NAR derivatives (39). These modifications can alter the properties and biological activities of NAR, potentially enhancing its effects or creating new therapeutic applications (40). NAR can trigger apoptosis in human cancer cells like CRC cells without displaying any toxic effects on normal cells at comparable dosage levels (41-44). It can activate signaling pathways such as caspase and p53 pathway, leading
to cell death and can further modify different signaling routes implicated in the onset and advancement of CRC. Furthermore, NAR suppresses CRC growth by inhibiting cyclooxygenase-1 (COX-1) (45-49). It can inhibit the activation of pathways like PI3K/Akt and MAPK, which are associated with cell survival, proliferation, and metastasis (48). Modified NAR derivatives (MNDs) such as 7-O-benzyl NAR (KUF-1) and 7-O-(m-methoxybenzyl) NAR (KUF-2) were synthesized and found to have significant apoptosis-inducing effects inside RKO cells of human CRC (44). The MNDs regulated the programmed cell death of RKO cells through intracellular reactive oxygen species (ROS) formation linked with the loss of mitochondrial membrane potential, activation of caspases, and prolonged ERK activation (44). Furthermore, studies have shown that NAR can effectively inhibit cell proliferation and induce cytoprotective autophagy in colonic carcinoma cells (50). The results suggest NAR could act as a potential new treatment for managing colon cancer and CRC (49).

The current study provided a thorough analysis of the potential chemopreventive effects of NAR and its derivatives on CRC by reviewing the current literature, with a focus on their cellular and molecular modes of operation.

**Overview of Naringenin**

**Molecular composition of Naringenin**

Naringenin (NAR), a trihydroxyflavanone (refer to Figure 1), scientifically termed 5,7-Dihydroxy-2-(4-hydroxyphenyl) chroman-4-one, is classified among phytochemicals known for their strong antioxidant and anti-inflammatory attributes (51). NAR's molecular structure is C15H12O5, featuring four hydroxyl (-OH) groups at the 5, 7, 4', and 3' positions on its B-ring (52, 53). This flavanone is found in various citrus fruits like oranges, grapefruits, and tomatoes (54, 55), and appears as a yellow crystalline solid with a melting point around 250-252°C (56). While NAR demonstrates limited water solubility, it dissolves in several organic solvents including ethanol and methanol (57, 58).

**Figure 1. Chemical structure of Naringenin**

2.2. Pharmacokinetic properties of Naringenin

2.2.1. Absorption

NAR has limited oral absorption because it is not very soluble in water and has limited ability to pass through the cells lining the intestines (59). Before absorption, NAR undergoes hydrolysis by β-glucosidase in the small intestine (60, 61). NAR undergoes absorption primarily in the small intestine, specifically within the duodenum, through passive diffusion. After being absorbed, NAR undergoes rapid conjugation resulting in the formation of glucuronide or sulphoglucuronide and attaching to serum albumin. It is then rapidly conveyed to various organs with high blood perfusion, including the kidney, heart, brain, liver, and spleen (62, 63). Additionally, the gut microbiota has been found to play a part in the metabolism and absorption of NAR, and some bacterial species can convert NAR into more bioavailable metabolites (64, 65).

2.2.2. Metabolism

NAR is metabolized in the colon through a fermentation process by the resident microbiota (66). This process results in the generation of diverse by-products, including hippuric acid, 3(3-hydroxyphenyl) propionic acid, 3(3,4-dihydroxyphenyl) propionic acid, or phloroglucinol (67, 68). Naringin, a glycoside derivative of NAR, can be converted into NAR by the liver enzyme beta-glucosidase through hydrolysis (69). NAR is primarily metabolized in the body through phase II metabolism, specifically glucuronidation and sulfation (70, 71). These processes involve the incorporation of glucuronic acid or sulfate moiety to NAR, leading to the production of NAR glucuronides and NAR sulfates, respectively (63). These metabolites are more water-soluble than NAR itself, facilitating their excretion from the body (72).

2.2.3. Excretion

The two main routes for the elimination of NAR from the body are the urinary and biliary pathways (73).
Once formed, NAR glucuronides and sulfates are typically eliminated through the bile into the gastrointestinal tract (74). From there, they can be further metabolized by gut bacteria or excreted in the feces. Some portion of these metabolites may also undergo enterohepatic circulation, where they are reabsorbed from the intestines back into the bloodstream and undergo further metabolism or excretion (75). A smaller fraction of NAR and its metabolites might be eliminated through the urine (76). However, the urinary excretion of NAR and its metabolites is generally considered to be relatively low compared to fecal excretion (77).

2.2.4. Toxicity

NAR is considered to have low toxicity, with an LD50 (lethal dose 50%) of 5000 mg/kg, indicating a high level of safety (78-81). It is generally considered safe when consumed in dietary amounts, such as those detected in citrus fruits and various botanical edibles (82). However, as with any compound, the toxicity of NAR can depend on the dose and duration of exposure (83). Studies have shown that NAR has a high LD50 value, indicating that it has low acute toxicity (78, 84). In animal studies, high doses of NAR did not cause significant adverse effects or mortality (85, 86). However, some studies have reported potential toxic effects of NAR at high doses, such as hepatotoxicity and reproductive toxicity (87, 88). NAR has relatively low bioavailability.

2.3. Drug delivery of Naringenin

To improve the bioavailability of NAR, various techniques and pharmacological formulations have been explored (54, 89). Nanoencapsulation involves encapsulating NAR within nanoparticles or liposomes to enhance its stability and solubility (90). This technique can protect NAR from degradation and improve its absorption and bioavailability (91). Solid dispersion involves dispersing NAR in a solid matrix, such as polymers or cyclodextrins, to enhance its solubility and dissolution rate (92). This technique can improve the release and absorption of NAR in the gastrointestinal tract. Complexation involves forming inclusion complexes of NAR with cyclodextrins or other complexing agents (93). This technique can enhance the solubility and stability of NAR, leading to improved bioavailability. Co-administration of NAR with absorption enhancers, such as piperine or quercetin, can improve its absorption and bioavailability (94). These enhancers can inhibit drug metabolism enzymes or enhance the transmembrane diffusion of NAR through the intestinal barrier (95). Lipid-centric formulations such as Self-emulsifying Drug Delivery Systems (SEDDS) have the potential to enhance the solubility and uptake of NAR, which is a poorly water-soluble compound (96). These formulations can improve the bioavailability of NAR by facilitating its dispersion and absorption in the gastrointestinal tract. Since NAR is not considered an essential nutrient, there are no official nutritional reference values (NRVs) or suggested daily intakes (SDIs) for this compound. However, research has explored the possible health advantages of NAR using doses ranging from 50 mg to 500 mg per day (97-99). These doses have been generally well-tolerated in human studies, with few reported adverse effects.

Naringenin and Colon Cancer

Colon cancer, a prevalent form of cancer that occurs in both males and females, has a higher likelihood of developing as individuals age. Several factors, such as family history, genetic mutations, inflammatory bowel disease, sedentary lifestyle, dietary choices, obesity, smoking, and alcohol consumption, may play a role in the risk elevation for colon cancer development (7, 8, 100). Symptoms of colon cancer can differ, but some common ones include unexplained weight loss, alterations in intestinal routines like frequent bowel movements or bowel sluggishness, abdominal distress or cramping, blood in the stool, exhaustion, and a sensation of incomplete bowel evacuation (101, 102). Screening for colon cancer is important for prompt detection and preventive measures. Standard screening techniques encompass colonoscopy, permitting the detection and removal of polyps, along with fecal-based assessments and diagnostic imaging examinations (103, 104). Therapeutic approaches for colon cancer depend on its stage and can include surgical intervention to excise the tumor, chemotherapeutic treatments, radiological therapy, targeted therapy, or a combination of these approaches (105, 106). The prognosis for colon cancer varies based on variables like the stage of the cancer, the individual’s overall health, and the effectiveness of treatment (107, 108). The chances of a successful outcome for individuals with colon cancer can be significantly improved through early detection.
Flavonoids are demonstrated to exert various impacts on cancer cells (Figure 2). Chronic inflammation is a significant factor that can promote the evolution and advancement of colon cancer, and flavonoids may help reduce inflammation and inhibit the proliferation of malignant cells (109-111). These compounds are known for their antioxidant capabilities, offering protection against the damage triggered by free radicals (112). Oxidative stress is a recognized factor in colon cancer genesis, and the antioxidative action of flavonoids might aid in thwarting or decelerating the disease’s advancement (65). Apoptosis, the body’s mechanism to remove defective or atypical cells (113), is induced by flavonoids in colon cancer cells, aiding in restraining their growth and enhancing cellular demise (114). Flavonoids also disrupt the cellular pathways that regulate cell multiplication and division, potentially suppressing the propagation of colon cancer cells (115), thereby impeding the cancer cells’ proliferation and dissemination. Research has explored the potential of flavonoids to counteract drug resistance in colon cancer therapies (115). Certain studies indicate that flavonoids can amplify the effectiveness of chemotherapy drugs by elevating cancer cells’ susceptibility, thus enhancing their potency in eradicating cancer cells (116-118).

The fermentation extract of NAR (FEN) is a product obtained from the fermentation of NAR by gut microbiota. After being digested, NAR is metabolized by the gut microbiota in the colon through a process of fermentation. This metabolism results in the production of by-products, including hippuric acid and phloroglucinol. These by-products exhibit antioxidative and anti-inflammatory characteristics. (36, 67). Bisphenol A (BPA), a prevalent chemical constituent, is extensively employed in fabricating polycarbonate polymers and epoxy resins. It is frequently present in food and beverage packaging, such as plastic bottles and canned foods, as well as in other consumer products, such as thermal paper receipts and dental sealants (119). BPA is known to have endocrine-disrupting properties, which means that it can disrupt the regular operation of hormones in the body. ERβ is a subtype of the estrogen receptor, which is a protein that binds to the hormone estrogen. ERβ is one of the two main types of estrogen receptors, with the other being ERα (120, 121). ERβ is found in various tissues throughout the body, including the colon. It has a part in mediating the effects of estrogen, a hormone involved in various physiological processes. ERβ is involved in regulating gene expression and communication routes crucial for cellular proliferation, differentiation, and apoptosis (122, 123). The p53 signaling route is pivotal in regulating cellular proliferation, differentiation, and programmed cell death. Often, it’s termed the "protector of the genetic code" because it has a vital part in preventing the formation of cancer by detecting and repairing DNA damage (124, 125). In research by Herrera et al., they showed that both NAR and its derivative FEN exhibited a reduction in the survival of HT-29 colon carcinoma cells. This phenomenon was noted upon singular administration of the compounds as well as in combination with BPA exposure. They also showed that NAR and FEN suppressed the metastasis induced by BPA in HT-29 cells. Additionally, the study examined the impacts of NAR on the expression of ERβ, miR-200c, miR-141, and genes engaged in the p53 signaling route in human colon carcinoma cells exposed to BPA. In addition, NAR can regulate the expression of genes associated with the p53 signaling route, including PTEN, TP73, and BBC3. These genes are pivotal in apoptosis and the control of the cell cycle (36).

In colon cancer, there is a dysregulation of the apoptotic route, resulting in a decreased rate of cell death. This allows cancer cells to survive and proliferate, contributing to tumor growth and progression. Apoptosis is influenced by genetic factors, and disruptions in the genes engaged in the apoptotic route can contribute to
the formation of colon carcinoma (126). For instance, alterations in the p53 gene, an oncogene inhibitor, can disrupt apoptosis and promote malignancy (127). Disruption of key apoptotic proteins, such as Bax, apaf-1, and caspase-9 can provoke oncogenic alteration and tumor development in colon cancer. These proteins contribute to the regulation of apoptosis and their dysfunction can contribute to the survival and growth of carcinoma cells (128). Diosmin (DS), a flavonoid glycoside, is found in various botanical origins, particularly in citrus fruits. It has been studied for its pharmacological activities and has shown various beneficial effects. DS exhibits anti-inflammatory traits, operates as a radical neutralizer, and has antimutagenic properties (129). DiNar is a combination of DS and NAR that was studied for its potential to synergistically induce cytotoxicity and inhibit inflammatory pathways in colon carcinoma cell lines (129). Zeya et al. conducted a study to examine the combined impact of DS and NAR on colon carcinoma cells. They found that the combination of these two compounds resulted in a combined effect, augmenting the cytotoxicity of the carcinoma cells. The researchers used the MTT assay to evaluate the cytotoxic effects and analyzed the data using CompuSyn software. Treatment with either NAR or DS led to substantial increases in apoptosis in HCT116 and SW480 colorectal carcinoma cell cultures compared to untreated control cells. Moreover, the apoptotic rate in the group treated with the combination of NR and DiNar was markedly elevated than in the groups treated with NR or DS solely. The investigation revealed that apoptotic indicators such as Bax, p53, caspase 3, caspase 8, and caspase 9 significantly increased in HCT116 and SW480 cells post NAR and DS exposure, relative to the controls. Moreover, these proteins’ expression levels were notably elevated in the DiNar group versus the NAR or DS solo treatments. Conversely, the anti-apoptotic protein Bcl-2 diminished in HCT116 and SW480 cells post NAR and DS application, compared to the controls. The study infers that combining DS and NR might be an effective strategy for colon cancer therapy (129).

6-CEPN, a small molecule compound, is also known as 6-C-(E-phenylethenyl) NAR. It is present in NAR-fortified fried beef. Treatment with 6-CEPN leads to inhibition of cell cycle progression in the G1 phase, impeding the proliferation of cancer cells progressing through the cell cycle and dividing (65). Autophagy is a cellular process that has an important part in preserving cellular homeostasis and survival. This protein degradation system is a dynamic process that is triggered in cells under different environmental challenges such as nutritional scarcity, oxygen deficiency, and exposure to anti-neoplastic therapies (50). The RAS cluster of diminutive GTP-binding proteins are critical modulators of cellular signaling cascades that regulate key processes including proliferation, differentiation, and survival (130). RAS proteins function as molecular regulators, alternating between an inactive GDP-bound state and an active GTP-bound state (131). Alterations in RAS genes can lead to perpetual RAS protein activation, fostering aberrant cellular signaling and contributing to the emergence and progression of diverse cancers (132). Zhao et al. observed that 6-CEPN impedes the proliferation of colon cancer cells, particularly in SW620 and HCT116 lines. This growth inhibition is effected through the induction of cell cycle arrest and necrotic cell demise. Furthermore, 6-CEPN treatment initiates autophagy in SW620 and HCT116 cells. The research indicates that 6-CEPN effectively suppresses RAS activation, an essential pathway in cell proliferation. This suppression hampers subsequent effector pathways, including c-Raf/mitogen-activated protein kinase (MEK)/extracellular signal-regulated kinase and phosphoinositide 3-kinase/AKT/mTOR pathways. The findings imply that 6-CEPN holds potential as an anticancer agent for colon cancer due to its capabilities to inhibit cell growth and trigger autophagy (50).

ATF3 is a member of the ATF/CREB family of transcription factors and is activated by various physiological and pathological stimuli. It plays a crucial role in gene regulation and is involved in several cellular processes, including apoptosis and cell cycle arrest (133). In the realm of oncology, ATF3 has emerged as a key molecular target for the apoptotic effects of many anti-cancer drugs in colon cancer cells. The activation of ATF3 is seen as a potential strategy for cancer prevention and therapy in human colon cancer (134). p38 MAPK, a signaling protein, is engaged in cellular responses to a variety of stress stimuli and is often referred to as p38. It is a member of the MAPK family and is instrumental in controlling cellular processes such as inflammation, cell differentiation, apoptosis, and stress response (135, 136). p38 MAPK is activated by phosphorylation due to various stress signals, including oxidative stress, cytokines, and growth factors. Upon
activation, p38 MAPK can phosphorylate and activate downstream targets, including transcription factors and other kinases, thereby regulating gene expression and cellular responses (137). The p38 MAPK signaling pathway is linked to a variety of physiological and pathological processes, including cancer, inflammation, and neurodegenerative diseases (138, 139). Song et al. discovered that NAR triggers apoptosis in human colon cancer cells. They specifically showed that NAR activates p38, leading to an increase in the transcriptional activation and protein level of ATF3. The expression of ATF3 mediated by NAR is partially responsible for apoptosis in human colon cancer cells. They also demonstrated that NAR induces the cleavage of PARP (a protein involved in DNA repair) in colon cancer cells in a dose-dependent manner, suggesting that the reduction in cell viability caused by NAR is due to apoptosis (134).

CDK2 is a key protein kinase that plays a central role in controlling cell cycle progression (140). It is an essential part of the cyclin-dependent kinase family, contributing significantly to the shift from the G1 phase to the S phase of the cell cycle (141). CDK2 associates with cyclin E to form a complex, which triggers its kinase activity, enabling it to phosphorylate downstream targets that are involved in DNA replication and cell cycle progression (142). The inhibition of CDK2 activity has been investigated as a potential therapeutic approach for cancer treatment (143). The investigators employed the clonogenic assay to assess the inhibitory effects of the synthesized NAR derivatives on HCT116 human colon cancer cells. In this study, Yoon et al. showed that NAR derivatives inhibit CDK2 activity through several potential mechanisms. First, the derivatives form hydrogen bonds with specific residues in the CDK2 protein, such as Glu12, Leu83, and His84, which are involved in the binding of the ligands. These hydrogen bonds aid in the fortification of the ligand-protein complex and potentially interfere with the enzymatic function of CDK2. Additionally, the derivatives also participate in hydrophobic interactions with several residues in CDK2, including Isoleucine10, Glycine11, Aspartic acid86, Glutamine131, and Leucine134. These hydrophobic interactions further contribute to the steadiness of the ligand-protein protein and may affect the conformation and activity of CDK2. Furthermore, the substantial substituents at the C-7 location of the derivatives may sterically hinder the binding of ATP or other substrates to CDK2, thereby inhibiting its enzymatic activity. Overall, the NAR derivatives inhibit CDK2 activity through a combination of hydrogen bonding, hydrophobic interactions, and steric hindrance effects. In this research, five NAR analogs were altered at the 7th position with different groups were synthesized. The derivatives are: N1 - modified with thiophenecarboxylate group, N2 - modified with methylbenzoate group, N3 - modified with isobutyrate group, N4 - modified with allyloxy group, and N5 - modified with phenyl carbonate group. This suggests that the NAR derivatives have stronger inhibitory activity against colon cancer cells compared to NAR. Moreover, the study uncovered that bulky substituents at the C-7 location of the flavanone framework improved the inhibitory effectiveness against HCT116 colon cancer cells. Consequently, these NAR derivatives demonstrate potential as prospective treatment modalities in colon cancer therapy (39).

Frydoonfar et al. indicated an analysis of the impact of Naringenin on the growth of HT-29 colorectal carcinoma cells. This research used a colorimetric assay to measure cell proliferation and found that NAR at concentrations greater than 0.71 mmol significantly inhibited cell growth in HT-29 colorectal carcinoma cell lines. The results of this study suggest that NAR possesses the capability to become a chemoprotective agent for colorectal carcinoma. This investigation underscores the significance of fruits and vegetables, notably citrus fruits, in mitigating the risk of various cancers, such as colorectal carcinoma. Notably, the influence of NAR on cellular proliferation varies with dosage. At reduced concentrations (0.02-0.09 mmol), NAR enhanced the growth of HT-29 colon cancer cells. Yet, at greater concentrations, NAR displayed a pronounced suppressive effect (144).

Colonic epithelial cells, which form the inner surface of the colon - the concluding section of the large bowel (145), are essential for the uptake of water, salts, and nourishment from the digested fare as it traverses through the colon (146). High multiplicity aberrant crypt foci (HAMCF) is characterized by the existence of abnormal crypts within the colon, with each focus comprising in excess of four abnormal crypts (147). Aberrant crypts are abnormal structures that can develop in the lining of the colon and are considered preneoplastic lesions, meaning they have the potential to progress to colon tumors (148). The presence of HAMCF is often used as an indicator of increased risk for colon cancer development (148). Leonardi
et al. investigated that dietary supplementation with apigenin and NAR, two citrus flavonoids, reduced the formation of HMACF inside the colonic tracts of rats exposed to azoxymethane (AOM), a carcinogen. Apigenin reduced the number of HMACF by 57%, while NAR reduced the number of HMACF by 51%. Additionally, NAR decreased cell proliferation in the colon by 32%. Both apigenin and NAR increased apoptosis of colon cells on the luminal surface. Moreover, studies have demonstrated that both apigenin and NAR possess the ability to impede the activity of specific enzymes involved in cell survival and proliferation, including COX-2 and iNOS (147). A synopsis of the study outcomes is depicted in Table 1.

**Naringenin and Colorectal Cancer**

Colorectal cancer (CRC) is a form of malignancy that begins in either the colon or the rectum, both of which are components of the large intestine in the digestive system. In most cases, CRC starts as benign growths referred to as polyps on the inner lining of the colon or rectum (149). Some of these polyps can eventually develop into cancerous tumors. If left untreated, CRC can invade nearby tissues and organs, and can also spread to distant sites through the bloodstream or lymphatic system (150). Studies have demonstrated that flavonoids exhibit anti-cancer effects by inhibiting oncogenic cell expansion and multiplication, triggering programmed apoptosis, and suppressing angiogenesis (151, 152). Moreover, some flavonoids have been found to have specific impacts on CRC cells, including the curtailment of cell multiplication and the activation of apoptotic pathways (Figure 3) (153, 154).

**Figure 3. Anticancer effects of NAR in CRC cells.**

Cyclin D1 is a vital protein in the control of cellular cycle progression, specifically the G1 phase (155). This protein is a member of the cyclin protein family, which promote the progression of the cell cycle by activating CDKs through binding. Cyclin D1 engages with CDK4 and CDK6 to create a complex that phosphorylates and neutralizes the retinoblastoma protein (Rb), permitting the cell to advance from the G1 phase to the S phase in the cellular cycle (156, 157). Elevated levels of cyclin D1 have been linked to various forms of cancer, such as CRC, breast cancer, and lymphoma. This suggests that focusing on cyclin D1 could be a potential approach for cancer treatment (158, 159). Song et al. carried out research to explore the inhibitory effect of NAR on human CRC cells. They found that NAR reduced the manifestation of cyclin D1, a protein involved in regulating the cellular cycle, which is frequently overexpressed in oncogenic cells. The downregulation of cyclin D1 was found to be facilitated through the activation of p38, a protein kinase involved in various cellular functions, including regulation of the cellular cycle and induction of programmed apoptosis. The study also found that NAR caused apoptosis in human CRC cells, indicating that it could serve as an effective chemopreventive and therapeutic agent for CRC (38).

Apoptosis acts as a natural defense mechanism against the development and advancement of CRC (160). It helps to eliminate damaged or abnormal cells, preventing their uncontrolled growth and potential transformation into cancer cells. Apoptosis helps maintain the equilibrium between cellular multiplication and apoptosis in the normal colonic epithelium (161). It ensures the turnover of cells and the removal of excess or unnecessary cells, contributing to the overall health and function of the colon (162). Necrosis represents a type of cellular demise that occurs as a result of acute injury or pathological processes (163). Unlike apoptosis, which is a programmed and controlled process, necrosis is characterized by uncontrolled cell death and the breakdown of cellular structures. Necrosis is associated with distinct morphological changes in cells. These changes include cell swelling characterized by plasma membrane disintegration, spillage of cell components, and consequent inflammation in adjacent tissues (113, 164). Abaza et al. discovered that NAR impedes the proliferation of colorectal cells via multiple pathways. Initially, it provokes a cessation in the cell cycle at the S- and G2/M-phases, leading to reduced cell proliferation. NAR also promotes cell cycle arrest and enhances apoptosis. It influences the gene expression related to both apoptosis and cell cycle control. Specifically, it diminishes the expression of genes such as cyclin-dependent kinases (Cdk4, Cdk7) and anti-apoptotic genes (Bcl2, c-IAP-2), while elevating the expression of pro-apoptotic genes (p18, caspases 3, 9, AIF, Bax) (165).

6-C-(E-phenylethenyl)-NAR (6CEPN) emerges as a novel, selective cyclooxygenase-1 (COX-1) antagonist, unearthed through advanced ligand-docking computational strategies. Synthesized via meticulous chemi-
cal protocols, its anti-neoplastic efficacy against colorectal carcinoma was scrutinized in vitro and in vivo (166). COX-1, pivotal in synthesizing prostaglandins — physiologically active eicosanoids — exhibits ubiquitous expression in assorted tissues, underpinning crucial homeostatic functions such as gastroprotective mechanisms, hemodynamic regulation, and thrombocyte aggregation (167, 168). Additionally, it contributes to inflammatory responses and thrombogenesis. Within oncological contexts, COX-1 sustains oncogenic attributes in colorectal carcinoma cells and the oncogenic transition in pre-malignant entities. Inhibitory modulation of COX-1 has shown efficacy in attenuating colorectal carcinoma proliferation (166, 169, 170). Li et al. discerned that 6CEPN impedes COX-1 enzymatic action, resulting in a pronounced inhibition of colorectal carcinoma expansion. Empirical evidence from in vitro and ex vivo assays confirmed 6CEPN’s specificity in COX-1 inhibition. The agent’s obstruction of COX-1 enzymatic function significantly impeded anchorage-independent neoplastic proliferation in colorectal carcinoma cells. Furthermore, in a 28-day colorectal carcinoma xenograft paradigm, 6CEPN demonstrated potent tumor growth suppression, devoid of overt systemic toxicological effects. These revelations underscore COX-1’s integral role in colorectal carcinoma pathogenesis, rendering 6CEPN a potentially formidable agent for colorectal carcinoma prevention (166). A concise encapsulation of these research findings is presented in Table 2.

**Conclusion**

Flavonoids, such as NAR, obtained from natural sources like fruits and vegetables, offer a potential approach for cancer treatment with reduced adverse effects. NAR, a type of flavonoid belonging to the flavanone subclass, is inherently found in citrus fruits such as oranges, grapefruits, and tomatoes. NAR has been investigated for its potential positive effects on health, such as its ability to act as an antioxidant and reduce inflammation. It has also been investigated for its anticancer effects, particularly in colon cancer and CRC cells. It can suppress cellular proliferation as well as inhibit the proliferation of oncogenic cells. NAR also acts as an ER modulator, specifically ERβ, which is crucial in inhibiting colon oncogenesis. Furthermore, pharmacological suppression of p38 mitogen-activated protein kinase prevented NAR from inducing ATF3 expression, activation of the ATF3 promoter, and apoptotic processes. These observations indicate that NAR triggers apoptosis in human colon cancer cells via p38-mediated activation of ATF3. In vitro, CDK2 binding assay was also performed, which showed that the NAR derivatives had better inhibitory activities on CDK2 compared to NAR. NAR downregulates the amount of cyclin D1 in CRC cells through multiple mechanisms. Additionally, NAR inhibited the growth of CRC cells in a manner dependent on both dosage and duration by halting the cellular cycle and inducing apoptotic cell death. Future clinical studies can provide more insights into the effectiveness and security of NAR as a prospective therapeutic choice for colon and CRC.

**Consent for publication**

Not applicable.

**Data Availability**

The data used to support the findings of this study are available from the corresponding author upon request.

**Conflict of interest**

The authors declare that they have no conflicts of interest.

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