Role of NRF2 in Colorectal Cancer Prevention and Treatment

Jiaxiang Li, BS[1] **, Dan Wang, MS**[1], **Yifei Liu, MS**[2],
and Yanhong Zhou, PHD[1]

Abstract

Nuclear factor erythroid 2-related factor 2 (NRF2) is a basic leucine zipper protein that participates in a complex regulatory network in the body. The activation of NRF2 can prevent and treat colorectal cancer (CRC). A variety of natural compounds can activate NRF2 to inhibit oxidative stress and inflammation to prevent the occurrence and development of CRC, inhibit the proliferation of CRC cells and induce their apoptosis. However, some studies have also shown that it also has negative effects on CRC, such as overexpression of NRF2 can promote the growth of colorectal tumors and increase the drug resistance of chemotherapeutic drugs such as 5-fluorouracil and oxaliplatin. Therefore, inhibition of NRF2 can also be helpful in the treatment of CRC. In this study, we analyze the current research progress of NRF2 in CRC from various aspects to provide new ideas for prevention and treatment based on the NRF2 signaling pathway in CRC.

Abbreviations

CRC, colorectal cancer; NRF2/NFE2L2, nuclear factor E2-related factor 2; bZIP, basic leucine zipper; sMaf, small musculoaponeurotic fibrosarcoma; ARE, antioxidant response element; Keap1, Kelch-like ECH associated protein; RXRa, recombinant retinoid X receptor alpha; NQO1, NAD(P)H dehydrogenase [quinone] 1; GST, glutathione S-transferase; HO-1, heme oxygenase-1; GSH-Px, glutathione peroxidase; SOD, superoxide dismutase; TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; IL-6, interleukin-6; COX-2, cyclooxygenase-2; ROS, reactive oxygen species; MACC1, metastasis-associated in colon cancer 1; NF-κB, nuclear factor kappa-B; MMP, matrix metalloproteinase; cIAPs, cellular inhibitors of apoptosis; c-FLIP, caspase-8/FADD-like IL-1beta-converting enzyme inhibitory protein; FADD, FAS-associated death domain; GST-A4, glutathione S-transferase A4; PRDX1, peroxiredoxin protein-1; IL-17A, Interleukin-17A; hTERT, human telomerase reverse transcriptase; PD-L1, programmed cell death receptor-ligand 1

Introduction

Colorectal cancer (CRC) is a common malignant tumor, with the third morbidity and the second mortality in the world, and the incidence is increasing year by year.[1] The occurrence of CRC leads to a reduced quality of life and significantly increases the risk of death, making CRC prevention and treatment extremely important. Nuclear factor E2-related factor 2 (NFE2L2/NRF2) is a basic leucine zipper protein that regulates the expression of antioxidant proteins. It is involved in a complex regulatory network and plays multiple roles in the regulation of metabolism, inflammation, autophagy, proteostasis,

[1] School of Pharmacy, Xianning Medical College, Hubei University of Science and Technology, Xianning, Hubei, China
[2] School of Stomatology and Ophthalmology, Xianning Medical College, Hubei University of Science and Technology, Xianning, Hubei, China

Corresponding Authors:
Yanhong Zhou, School of Pharmacy, Xianning Medical College, Hubei University of Science and Technology, Xianning, Hubei, China.
Email: zyh@hbust.edu.cn

Yifei Liu, School of Stomatology and Ophthalmology, Xianning medical college, Hubei University of Science and Technology, Xianning, Hubei, China.
Email: liuyifei@hbust.edu.cn.

mitochondrial physiology, and immune responses.[2] Under normal conditions, the activation of NRF2 can prevent the development of CRC, including reducing the proliferation of colon cancer cells and increasing the apoptosis of colon cancer cells. However, overexpression of NRF2 may also have negative effects, by creating the best environment for cell growth to promote the survival of normal cells and cancer cells, protect tumor cells from oxidative stress, chemotherapeutic drugs, and radiotherapy, and promote tumor development.[3] In this paper, we summarize the complexity of NRF2 regulation in CRC and clarify the significance of NRF2 in CRC prevention and treatment through different perspectives.

## Structure and Function of NRF2

NRF2 is encoded by the NFE2L2 gene and belongs to the Cap'n'Collar subfamily of basic leucine zipper (bZIP) transcription factors, which contains nuclear factor 2 (erythroid-derived 2 NFE2) and NRF1, NRF2, and NRF3.[4] NRF2 has seven conserved NRF2-ECH (Neh) homologous domains and has different functions to control NRF2 transcriptional activity. BZIP in the Neh1 domain is heterodimerized with small Musculo aponeurotic fibrosarcoma (sMaf) oncogene homolog K, G, F, and other bZIP proteins to identify antioxidant response elements (AREs) to activate gene transcription, while the Neh2 domain contains ETGE and DLG motifs, which specifically interact with the Kelch domain of

Kelch-like ECH-related protein 1 (Kelch-like ECH associated protein, Keap1) to ubiquitinate and degrade NRF2.[5] The Neh3-5 structural domain functions as a transcriptional activation structural domain by binding to various components of the transcriptional machinery.[6] The Neh6 structural domain contains 2 redox-independent degraders, DSGIS and DSAPGS, which bind to the E3 ubiquitin ligase β-transducing repeat protein and mediates the degradation of NRF2 in oxidatively stressed cells.[7] The Neh7 structural domain mediates the interaction with the Recombinant Retinoid X Receptor Alpha (RXRa), which inhibits NRF2 activity.[8]

Under normal conditions, NRF2 binds to Keap1 through its 2 motifs (ETGE and DLG). Keap1 promotes the ubiquitination of NRF2 in the cytoplasm and keeps it at a low level in the cytoplasm.[9] When exposed to oxidative stress or using an NRF2 activator, NRF2 is dissociated from Keap1 binding and then translocated to the nucleus, where it transcribes with sMaf protein polymer and ARE gene to activate the expression of multiple genes[10] (Figure 1). These include glutathione S-transferase (GST), NAD(P)H dehydrogenase [quinone] 1 (NQO1), heme oxygenase-1 (HO-1), glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD).[11]

The Role of NRF2 in CRC

Studies have shown that long-term chronic inflammation leads to increased levels of pro-inflammatory cytokines in the colonic
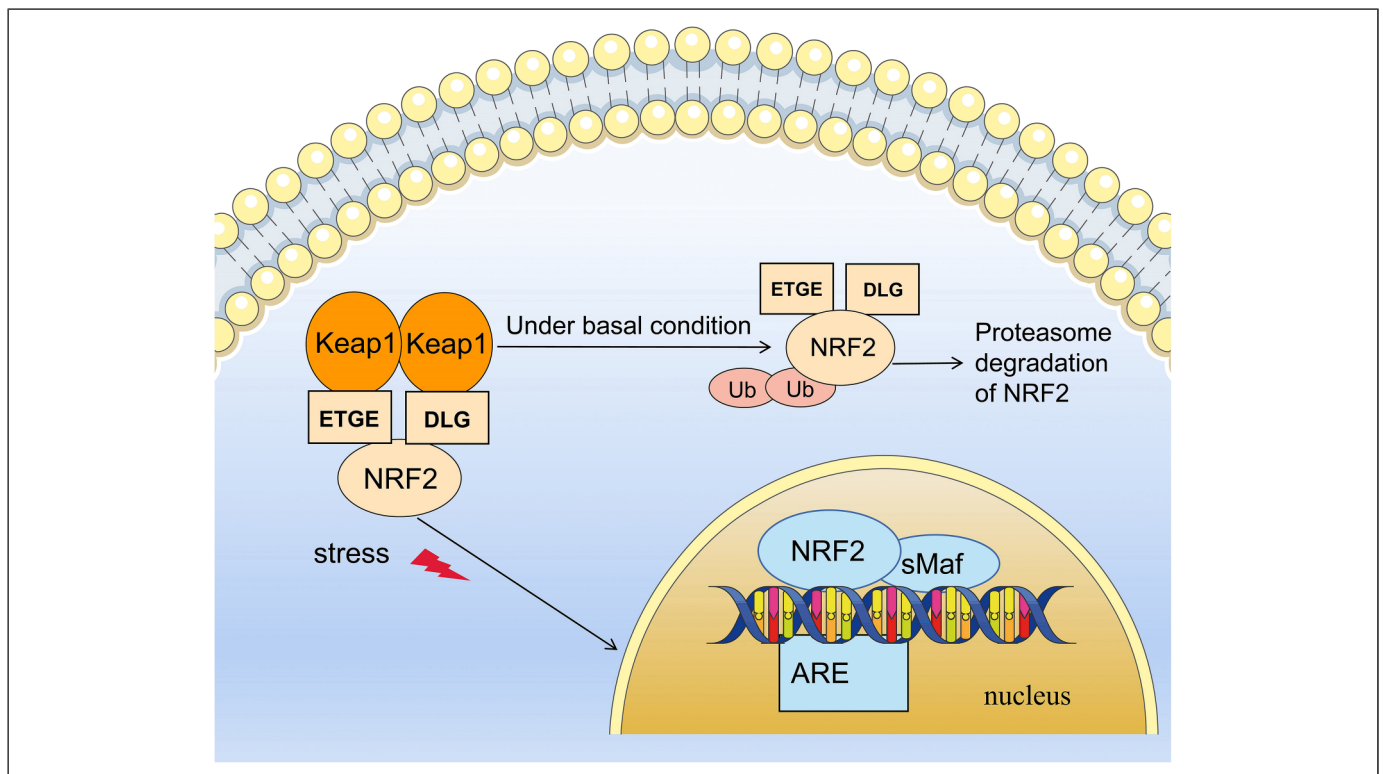
Figure 1. Under normal conditions, NRF2 binds to Keap1 through its two motifs (ETGE and DLG), and Keap1 promotes the ubiquitination of NRF2 in the cytoplasm. Under oxidative stress, NRF2 dissociates from Keap1 and translocates to the nucleus, which activates the expression of many genes with sMaf protein polymer and ARE gene transcription.

Abbreviations: NRF2, nuclear factor erythroid 2-related factor 2; ARE, antioxidant response element.

mucosa, such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), etc, thereby promoting inflammation and cancer.[12] IL-6 promotes tumor growth and inhibits apoptosis by activating the JAK/STAT signaling pathway. IL-1β and TNF-α cause cyclooxygenase-2 (COX-2) to be overexpressed in the early stages of carcinogenesis and also lead to angiogenesis, cell proliferation, and apoptosis. Pro-inflammatory cytokines are also able to promote the production of reactive oxygen species (ROS) and reactive nitrogen intermediates, both of which cause DNA damage by way of oxidative stress, thereby causing the development of cancer.[13] In addition, after stimulation by TNF-α, the expression of metastasis-associated in colon cancer 1 (MACC1), which induces cancer cell proliferation and increases metastasis, is significantly increased at both gene and protein levels.[14] Therefore, controlling inflammation is an effective means to inhibit the occurrence and development of CRC.

Nuclear factor kappa-B (NF-κB) is a central regulator of inflammation that regulates a variety of biological processes. Upon inflammation, NF-κB is activated, leading to the transcription of various cytokines such as COX-2, TNF-α, IL-1β, and IL-6.[15] TNF-α and IL-1β stimulate matrix metalloproteinase (MMP) production, leading to the extracellular matrix and mucosal damage. In colonic lamina propria fibroblasts, activation of NF-κB can lead to increased expression of IL-8, IL-6, and chemokines, which are attracted by neutrophils to sites of inflammation and play a key role in their development, promoting tumor development through tissue infiltration and metastasis.[16] Tumor cell growth requires the continuous formation of new blood vessels, and IL-6 and TNF-α has been shown to promote angiogenesis. Persistent

activation of NF-κB also inhibits the transcription of apoptosis-related genes such as Cellular inhibitors of apoptosis (cIAPs), Caspase-8/FADD-like IL-1beta-converting enzyme inhibitory protein (c-FLIP) and members of the bcl2 family (eg A1/BFL1 and bcl-xl).[17] In conclusion, activation of NF-κB promotes tumorigenesis by promoting cell proliferation and angiogenesis, inhibiting apoptosis, and promoting cell invasion and metastasis.

Therefore, preventing the activation of NF-κB and the expression of downstream cytokines is an effective means to resist the occurrence and development of tumors. The activation of NRF2 leads to the expression of HO-1, and the upregulation of HO-1 expression decreased the release of proinflammatory cytokines such as TNF-α, IL-6, etc.[18] HO-1 converts heme into biliverdin and CO, which play an important role in cytoprotection against oxidative stress.[19] Low concentration of CO can attenuate NF-κB p65 nuclear translocation.[20] Thus reducing the release of proinflammatory cytokines. Therefore, inflammation induced by oxidative stress leads to further activation of NF-κB and excessive production of cytokines, and the activation of NRF2 plays an important role in interrupting this cycle (Figure 2).[21]

In AOM/DSS-induced colorectal cancer mice, NRF2 knockout mice were more likely to develop colorectal cancer than wild-type mice. The main factors for this increased susceptibility were impaired antioxidant/detoxification mechanisms and increased pro-inflammatory arachidonic acid metabolism, and the lack of NRF2 significantly reduced the diversity of colon cancer gene expression.[22,23] Lee et al found that the activation of the NRF2 signaling pathway by CyCl can reduce proliferation and colony formation in colon cancer cells by inhibiting the NF-κB signaling pathway and inducing apoptosis.[24] Shukla et al[25] indicated that
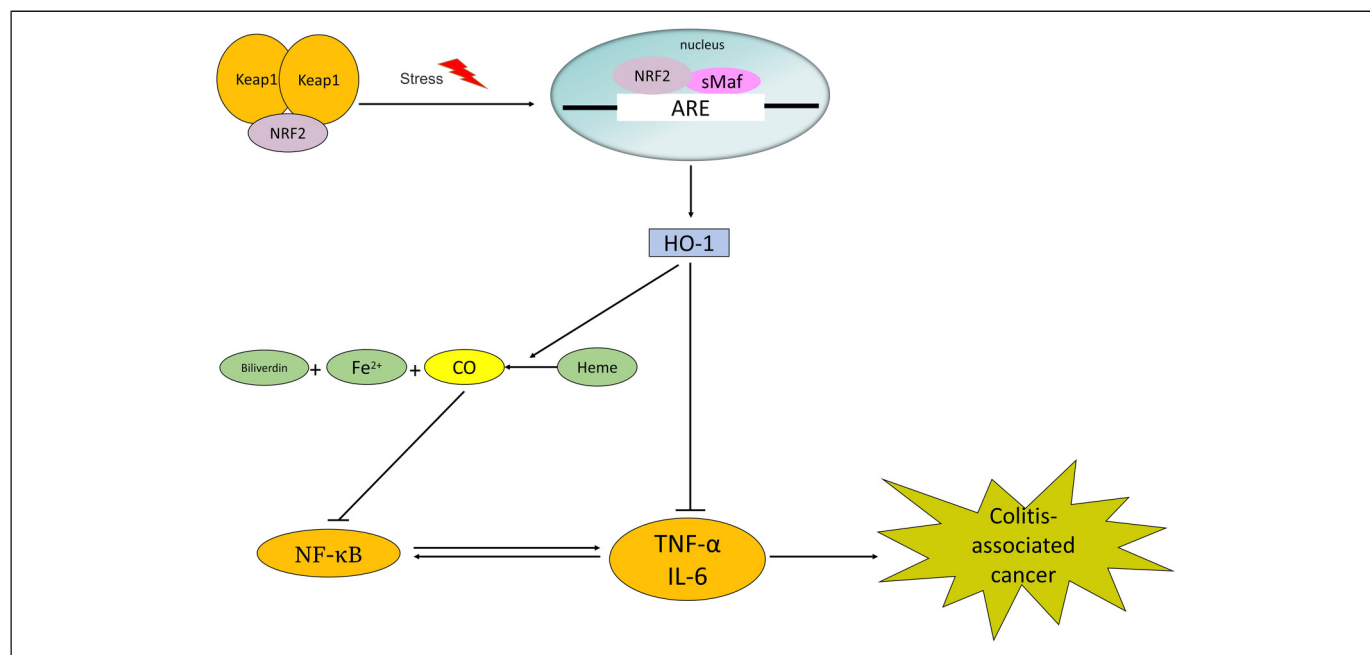
Figure 2. When stimulated by oxidative stress, NRF2 transcripts to activate the expression of HO-1, and HO-1 can inhibit TNF-α and IL-6. And it can induce heme decomposition to produce CO, which can further inhibit the production of TNF-α and IL-6 by NF-κB, and finally prevent the occurrence and development of CRC.
Abbreviations: NRF2, nuclear factor erythroid 2-related factor 2; CRC, colorectal cancer.

AR inhibitor prevents CRC growth by increasing mitochondrial biogenesis via increasing the expression of NRF2/HO-1/ AMPK/p53 and decreasing the mitochondrial DNA damage. Activation of NRF2 also affects matrix metalloproteinase (MMP). The NRF2/HO-1 axis inhibits MMP-7 in human intestinal epithelial cells. Expression of MMP-3 induced by oxidative stress is elevated in NRF2-deficient mice. In contrast, MMP-3 levels were also elevated in patients with inflammatory bowel disease, which may suggest that NRF2 has an inhibitory effect on MMP-3, resulting in a therapeutic effect on inflammatory bowel disease.[26] In addition, NRF2 induces the expression of biphasic enzymes and reduces the risk of tissue carcinogenesis.

Higher NRF2 levels were positively correlated with antioxidant enzymes such as glutathione S-transferase A4 (GST-A4) and peroxiredoxin protein-1 (PRDX1), and with the Interleukin-17A (IL-17A) was negatively correlated.[27] In summary, the development of CRC can be inhibited by activating NRF2. In recent years, most studies have shown that some natural compounds can treat and prevent CRC by activating NRF2 (Table 1).

Negative Role of NRF2 in CRC

However, studies have found that NRF2 can also promote cancer. Tao et al[38] treated mice with NRF2 regulators to test

Table 1. Natural Compounds in the Prevention and Treatment of Colorectal Cancer.

Chemical compound	Cell types/animal model	Result	Reference
Luteolin	AOM/DSS mouse model HCT116 cells HT29 cells	luteolin upregulated NRF2 and UDP-GT and GST levels and increased GST-a and GST-μ expression, thereby inhibiting tumorigenic development during AOM/DSS-induced CRC. It also inhibited the proliferation and transformation of HCT116 cells and HT29 cells by increasing the expression of NRF2.	28,29
Sulforaphane (SFN)	HT-29 cells SW480 cells	SFN increases nuclear translocation of NRF2, inhibits cell proliferation and colony formation, and induces apoptosis in colon cancer HT-29 and SW480 cells.	30
Shaoyao decoction (SYD)	AOM/DSS mouse model HT-29 cells	By activating the NRF2 pathway, it can up-regulate the expression of mRNA and proteins such as NRF2 and its downstream HO-1, NQO-1 in vivo and in vitro, effectively enhance the antioxidant capacity of the body, and reduce the expression of inflammatory factors such as NF-κB, TNF-α, and IL-1β, so as to prevent the occurrence of colitis-associated CRC.	31
Ginnalin A	HCT116 cells SW480 cells SW620 cells	Ginnalin A showed a good inhibitory effect on CRC cells (HCT116, SW480, and SW620), significantly reducing cell proliferation. Ginnalin A inhibited tumor proliferation by inducing cell cycle arrest in the S phase. And it upregulated the expression levels of mRNA and protein of NRF2-associated antioxidant genes HO-1 and NQO1.	32
Digitoflavone	AOM/DSS mouse model Caco-2 cells	Digitalis ketones induce the G2 phase cell cycle arrest, inhibit angiogenesis and down-regulate NF-κB expression, and increase NRF2 expression, nuclear translocation, and antioxidant enzyme expression. In addition, digitalis ketones reduce H_2O_2-induced oxidative stress and cell death through the p38 MAPK-NTF2/ARE pathway. In vivo studies showed that 50 mg/kg of digitalis significantly reduced the incidence, number, and size of AOM-DSS-induced tumors.	33
Tagitinin C	HCT116 cells	Tagitinin C inhibits the growth of CRC cells including HCT116 cells and induced an oxidative cellular microenvironment resulting in ferroptosis of HCT116 cells. Tagitinin C-induced ferroptosis was accompanied by the attenuation of glutathione (GSH) levels and an increased in lipid peroxidation. Mechanistically, tagitinin C induced endoplasmic reticulum (ER) stress and oxidative stress, thus activating nuclear translocation of NRF2. HO-1 expression increased significantly with the treatment of tagitinin C. Upregulated HO-1 led to an increase in the labile iron pool, which promoted lipid peroxidation, meanwhile tagitinin C showed a synergistic anti-tumor effect together with erastin.	34
Wogonin	AOM/DSS mouse model HTC116 cells	Wogonin inhibits the expression of NF-κB, IL-6, and IL-1, promotes the expression of NRF2, reduces the incidence of inflammatory-related colorectal cancer tumors, and inhibits the growth of HCT116 cells.	35
Cylon cinnamon	AOM/DSS mouse model	CA increases the expression of NRF2 in mouse colonic epithelial cells, thereby inhibiting colorectal carcinogenesis in the AOM/DSS mouse model.	36
Quercetin	Rats with DMH induced colon carcinogenesis.	quercetin supplementation effectively reversed DMH-mediated oxidative stress and DNA damage by targeting the NRF2/Keap1 signaling pathway.	37

the role of NRF2 in cancer. The results showed that the activation of NRF2 could prevent the occurrence of chemically induced cancer. However, whether caused by chemical or genetic cancer, can promote the progression of existing tumors. If the tumor has already occurred, the continued development of the tumor can be prevented by inhibiting NRF2. High expression of human telomerase reverse transcriptase (hTERT) is associated with the progression of CRC and negatively correlates with survival in CRC patients. Previous studies have shown that hTERT reduces ROS levels in cancer cells and accelerates cancer progression. hTERT promotes CRC proliferation and migration through upregulation of NRF2 expression by recruiting the transcription factor YBX1 to activate the NRF2 promoter.[39] Evans et al[40] applied tissue microarrays and found that, relative to normal colon tissue, NRF2 in primary CRC and metastatic cancer tissues were highly expressed ($P < 0.01$), and NRF2 expression in matched primary and metastatic specimens was positively correlated. In vitro experiments showed that NRF2 siRNA and NRF2 inhibitor Brusatol reduced cell survival and sensitized cells by irinotecan. In addition, Brusatol was effective in inhibiting CRC development in subcutaneous and allograft CRC mice. Studies by Torrente et al[41] have shown that NRF2 is overactivated in colorectal tumors, which is associated with poor prognosis. These studies have shown that in existing CRC, the activation of NRF2 promotes the development of cancer.

The activation of NRF2 is associated with resistance to chemotherapy. 5-Fluorouracil is the standard drug for CRC chemotherapy. Zhao et al[42] found that resistance to 5-fluorouracil treatment was associated with higher ROS production, upregulation of NRF2, and increased expression of HO-1 in CRC cell lines. Programmed Cell Death Receptor-Ligand 1 (PD-L1) is one of the downstream targets of the NRF2. This molecule has some beneficial effects on tumor growth by suppressing the immune system. Payandeh et al[43] examined the expression of NRF2, PD-L1, and CD80 in tumors and cut edge tissues of CRC patients. The role of the NRF2-PD-L1 axis in promoting oxaliplatin resistance was investigated. The results showed that NRF2 and PD-L1 mRNA expression was significantly higher in tumor tissues than in tangential margin tissues. PD-L1mRNA expression levels were also elevated in drug-resistant cells. While NRF2 expression was decreased in SW480/RES cells, NRF2 expression was increased in LS174T/RES cells. Inhibition of NRF2 by siRNA treatment in SW480/RES and LS174T/RES cells decreased the IC50 values of oxaliplatin. Inhibition of NRF2 caused a significant increase in oxaliplatin-induced apoptosis and a decrease in migration in SW480/RES cells. Effective inhibition of the NRF2-PD-L1 signaling pathway could be a new way to improve the efficacy of oxaliplatin in the treatment of CRC. Therefore, in the process of traditional chemotherapy, inhibition of NRF2 is an effective means to reduce drug resistance, and many studies have shown its effectiveness (Table 2).

Discussion

NRF2 participates in various physiological processes and in complex regulatory mechanisms in cancer and plays a key role in intestinal physiological function and the prevention and treatment of CRC. In recent years, NRF2 has received more and more attention. In inflammation-related CRC, a large number of studies have shown that the expression of NRF2 and the activation of downstream genes can protect intestinal cells from oxidative stress, inflammation, and other damage, and many studies have proved that lack of NRF2 is more likely to suffer from CRC. Therefore, activating NRF2 is effective in preventing CRC. As activators of NRF2, many natural compounds can effectively prevent the occurrence and development of inflammation-related colorectal cancer. In a word, the activation of NRF2 is very important for the prevention of CRC, and further study on the preventive effect of activating NRF2 on CRC should be paid attention to in the future.

Table 2. Different Types of Compounds/Genes Reduce Chemotherapeutic Drug Resistance by Inhibiting NRF2 in CRC.

Compound/gene	Result	Reference
FoxO3	FoxO3 can reverse CRC resistance to 5-FU by inhibiting the NRF2/TR1 signaling pathway and increasing intracellular levels of reactive oxygen species	44
Quinacrine(QC)	Treatment of mouse CRC xenografts with a combination of QC and 5-FU inhibited tumor growth more effectively than QC or 5-FU alone. QC enhances the sensitivity of CRC cells to 5-FU under hypoxic conditions by enhancing JNK1-dependent degradation of NRF2.	45
Trigonelline	Trigonelline inhibits NRF2 and ARE expression in CRC cells, and trigonelline micelles increase oxaliplatin-induced apoptosis in an NRF2/ARE-dependent manner.	46
RV-59	A nude mouse xenograft tumor model showed that RV-59 efficiently suppressed tumor growth induced by transplanted NLS-mutated NRF2-transfected shNRF2-HCT116 stable clones without affecting the bodyweight of the nude mice over the 37-day experimental period.	47
Manuka honey (MH)	Downregulation of transcription factor (NF-κB and NRF2) and antioxidant enzyme activity (SOD, catalase, glutathione peroxidase, and glutathione reductase) and expression (SOD, catalase, and HO-1) were more evident after the combined treatment, leading to more cell death by oxidative stress.	48
Dihydromyricetin (DMY)	DMY restored chemosensitivity (OXA and VCR) by inhibiting both MRP2 expression and its promoter activity in HCT116/OXA and HCT8/VCR cell lines. Furthermore, DMY could inhibit NF-κB/p65 expression, reducing NF-κB/p65 translocation to the nucleus to silence NRF2 signaling, which is necessary for MRP2 expression.	49

On the contrary, the negative role of NRF2 in CRC should not be ignored, because the expression of NRF2 can lead to drug resistance to some chemotherapeutic drugs, when using traditional chemotherapy to treat CRC, we should also inhibit NRF2 to reduce drug resistance to chemotherapy to improve the efficacy of chemotherapy. The overexpression of NRF2 in colorectal cancer may also promote the proliferation and migration of CRC. Inhibition of its overexpression in CRC may be effective in the treatment of CRC. However, there are few studies on NRF2 inhibitors in the treatment of CRC, so more studies on NRF2 inhibitors in CRC are needed in the future to clarify the role of NRF2 in CRC.

Declaration of Conflicting Interests

Funding

ORCID iD

Jiaxiang Li 🔟 https://orcid.org/0000-0002-8769-0440

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