

The Dual Role of NRF2 in Colorectal Cancer: Targeting NRF2 as a Potential Therapeutic Approach

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Abstract: Colorectal cancer (CRC), as the third most common bisexual cancer worldwide, requires urgent research on its underlying mechanisms and intervention methods. NRF2 is an important transcription factor involved in the regulation of redox homeostasis, protein degradation, DNA repair, and other cancer processes, playing an important role in cancer. In recent years, the complex role of NRF2 in CRC has been continuously revealed: on the one hand, it exhibits a chemopreventive effect on cancer by protecting normal cells from oxidative stress, and on the other hand, it also exhibits a protective effect on malignant cells. Therefore, this article explores the dual role of NRF2 and its related signaling pathways in CRC, including their chemical protective properties and promoting effects in the occurrence, development, metastasis, and chemotherapy resistance of CRC. In addition, this article focuses on exploring the regulation of NRF2 in CRC ferroptosis, as well as NRF2 drug modulators (activators and inhibitors) targeting CRC, including natural products, compounds, and traditional Chinese medicine formulations.

Keywords: NRF2, CRC, ferroptosis, pharmacological modulators

Background

NRF2 (nuclear factor (erythroid-derived 2)-like 2) is a 66-kDa transcription factor which contains a Cap'n'Collar (CNC) structure.¹ It belongs to basic-leucine zipper transcription factor family, is an effective transcriptional activator that plays a core role in protecting cells from oxidative and electrophilic stress.² The activity of NRF2 is strictly regulated by Kelch like ECH related protein 1 (KEAP1), the main negative regulator of NRF2. KEAP1 belongs to the Cul3 ubiquitin ligase family and mediates the polyubiquitination and proteasomal degradation of NRF2 protein under basic (stress free) conditions, which is the main way that KEAP1 regulates NRF2. Under cellular stress conditions, the structural integrity of the KEAP1-CUL3 ligase complex is disrupted, leading to a decrease in ubiquitination activity and an increase in cellular NRF2. Unbound NRF2 translocate to the nucleus and binds to the antioxidant response element (ARE) sequence to regulate the transcription of gene suites, including intracellular redox control, metabolic pathways, autophagy, and drug transport.³

In the past, the NRF2 signaling pathway was often considered to have the ability to act as a tumor suppressor. NRF2 enables cells to resist stresses such as carcinogens and inhibits tumor growth and progression through anti-inflammatory and antioxidant effects. It also plays an important role in protecting intestinal integrity by regulating pro-inflammatory cytokines and inducing Phase II detoxification enzymes. For example, remodelling the balance of M1/M2 subpopulations in the intestinal milieu can alleviate intestinal inflammation and prevent secondary colorectal carcinogenesis through NRF2-dependent macrophage reprogramming.⁴ In contrast, α -tocopherol quinone plays an important role in preventing CRC by consistently enhancing the intestinal epithelial tight junction barrier through NRF2-mediated inhibition of CLDN2 expression by STAT3 inhibition.⁵ However, recent studies have gradually found that the NRF2 pathway plays

a role in some tumors, including colorectal cancer, in an “oncogene” like manner. Promoting cell survival, resistance to radiation, chemotherapy, and metabolic disorders, and is associated with chemotherapy resistance and poor prognosis in CRC cells. At the same time, NRF2 maintains the proliferation and invasion of cancer cells during the CRC carcinogenesis stage by metabolizing reprogramming, inhibiting cancer cell apoptosis, and enhancing the self-renewal ability of cancer stem cells. More importantly, NRF2 is widely involved in iron, lipid, amino acid, and glucose metabolism and plays an extremely important role in the regulation of ferroptosis.⁶ Under oxidative stress conditions, nuclear translocation of NRF2 activates downstream gene expression, leading to increased expression of antioxidant proteins, which reduces ROS and the labile iron pool (LIP) to prevent additional production of ROS to block ferroptosis. In addition, NRF2 alters iron homeostasis by increasing iron storage and its flux into and out of the cell. The intracellular iron storage protein ferritin, including ferritin heavy chain (FTH) and ferritin light chain (FTL), segregates excess free iron in protein cages, thereby limiting redox conversion of iron. Thus, NRF2 has considerable potential to regulate ferroptosis in CRC cells, which may serve as a new avenue for CRC therapy.

The Expression of NRF2 in CRC Tissues

CRC is the third most common cancer worldwide.⁷ Over 1.9 million new CRC cases and 930000 deaths were estimated in 2020. The burden of CRC is projected to increase to 3.2 million new cases and 1.6 million deaths by 2040 with most cases predicted to occur in high or very high HDI countries.⁸ The occurrence of CRC is a multi-step process that involves multiple genetic changes. This process is usually divided into three stages: initiation, promotion, and progression, and has various biological characteristics,⁹ such as cell transformation, genomic instability, excessive proliferation, immortalization, angiogenesis, epithelial mesenchymal transition, and metastasis. NRF2 often plays an undeniable role in these processes and the characteristics exhibited by CRC.

NRF2 is specifically elevated in CRC tissue, significantly elevated at both mRNA and protein levels, and positively correlated with tumor size, lymph nodes, distant metastasis, and smoking status.¹⁰ During the progression of adenomas to cancer, multiple genes including NRF2 and genes encoding cell cycle pathway components undergo changes.¹¹ NRF2 gradually increases in the normal mucosal adenoma cancer direction and only shows significantly higher expression in CRC with rapid inflammatory peritumoral response, but does not show any correlation with tumor invasiveness characteristics.¹² However, interestingly, research by Professor Elena V Knatko's team has shown that NRF2 activation does not affect adenoma development in a mouse model of CRC, and NRF2 activation is unlikely to affect the early stages of CRC development.¹³

The expression of NRF2 exhibits significant heterogeneity based on the clinical staging and prognosis of CRC,¹⁴ making it the most promising biomarker for identifying poorly prognosis CRC. A metagene based study showed that NRF2 is a consistent and robust prognostic biomarker for all stages of CRC.¹⁵ High activity of NRF2 suggests poor prognosis in patients, especially high levels of nuclear NRF2 suggest poor prognosis.¹⁶ The cytoplasmic localization of NRF2 (cNrf2) expressed in CRC has a greater impact on tumor invasion and 5-FU resistance than nuclear localization (nNrf2), which can promote CRC invasiveness by upregulating PSMD4.^{17,18}

In addition, KEAP1 downregulates its mRNA levels through epigenetic modification and interacts with NRF2 to increase CRC risk.¹⁹ The role of NRF2 related transcription factor heme oxygenase 1 (Hmox1) in the growth and spread of CRC cannot be ignored too. A study showed that the ratio of Hmox1/ NRF2 mRNA levels in tumor tissues of distant metastatic subjects was significantly increased compared to non-metastatic patients.²⁰ And as a risk factor for CRC among workers, the promotion effect of red meat diet on CRC can be partially explained by initiating positive selection of precancerous cells through NRF2 dependent antioxidant response.²¹

The Complex Regulation of NRF2 in CRC

Not only is the abnormal expression of NRF2 itself, but the activation of NRF2 related signaling pathways is also closely related to the initiation, progression, and metastasis of CRC, and even treatment resistance. The involved pathways include classic anti-inflammatory and antioxidant pathways, as well as metabolic and ferroptosis signaling pathways that have gradually been emphasized in recent years. Therefore, here we summarize the main signal pathways regulated by NRF2 in CRC, and analyze its two-sided role in CRC.

NRF2 Mediated Chemoprevention Effects in CRC: Anti-Inflammatory and Antioxidant

Inflammation and oxidative stress play crucial roles in the occurrence and progression of various diseases, including CRC.^{22,23} Chronic OS can lead to oxidation of biomolecules (nucleic acids, lipids, and proteins) or activation of inflammatory signaling pathways, resulting in activation of several transcription factors or dysregulation of gene and protein expression, ultimately leading to tumor development or cancer cell survival.²⁴

As is well known, NRF2 is a classic stress transcription factor that maintains cellular homeostasis and controls the expression of antioxidant, cell protective, and detoxifying enzymes. The balance between KEAP1 and NRF2 is crucial for maintaining cellular homeostasis, and the KEAP1/NRF2 axis is recognized as the central node for cell defense and survival pathway crosstalk.²⁵ Under no pressure, NRF2 forms a homologous complex with KEAP1, which coexists in the cytoplasm to facilitate ubiquitination of NRF2. When oxidative or electrophilic stress exists, the oxidation of cysteine residues in KEAP1 interrupts the ubiquitination process of NRF2. Then NRF2 translocates to the nucleus, forms dimers with small Maf proteins, and binds to ARE to activate downstream gene expression, exerting antioxidant effects. Among them, NRF2 induced expression of phase II detoxifying enzymes can reduce the risk of tissue cancer and prevent early inflammation mediated tumor occurrence.²⁶ Meanwhile, the role of the KEAP1/NRF2 signaling axis in gastrointestinal development, anti-inflammatory, and antioxidant stress has been confirmed.²¹ Therefore, KEAP1/NRF2 has a protective effect in the early stage of CRC, and its inhibitory carcinogenic effect may be related to activating the antioxidant defense system, regulating inflammatory mediators to weaken oxidative stress.²⁷

The KEAP1-NRF2-ARE pathway is one of the most important defense mechanisms against oxidative and/or electrophilic stress,²⁸ which inhibits the development of acute/chronic colitis by inhibiting inflammation and preventing oxidative stress-induced cell damage.^{29,30} In HCT116 cells, the activated KEAP1/NRF2 pathway can upregulate SLC7A11 expression and glutathione levels, promote lipid peroxidation and protein oxidation, leading to mitochondrial damage and ultimately iron dependent cell death.²³ Meanwhile, activation of the KEAP1-NRF2-ARE pathway showed protective effects in AOM/DSS induced CRC mice.³¹ This indicates that the activation of the KEAP1-NRF2-ARE signaling pathway has a preventive effect on colitis related CRC. In addition, NRF2 upregulates the expression of the phase II detoxification enzyme glutathione S-transferase (including GST – α , μ), Protecting cancer progression during AOM induced CRC.³¹ And targeting the KEAP1/NRF2 signaling pathway can effectively reverse DMH mediated oxidative stress and DNA damage in rats.³² Specifically, the KEAP1/NRF2 signaling pathway activates major antioxidants (including enzymatic and non-enzymatic systems such as GSH, vitamin C, and E) to reduce ROS mediated cell damage and activate the transcription of phase II cell protective enzymes to upregulate intestinal barrier function, thereby playing a chemoprevention role in CRC.^{32–34}

ARE and HO-1 are classic antioxidant factors downstream of NRF2, commonly believed to protect cells from cancer and slow down cancer development by neutralizing ROS or carcinogens.³⁵ In vivo studies have shown that 50 mg/kg digitalis can significantly reduce the incidence, number and size of AOM-DSS induced tumors in mice, and reduce H₂O₂ induced oxidative stress and cell death through p38 MAPK-NRF2/ARE pathway.³⁶ And the fermented ileal fluid rich in raspberry can drive upregulation of the cell protective NRF2-ARE pathway to reduce DNA damage in normal colon cells.³⁷ In addition, activating the NRF2/HO-1 axis can specifically exert anti-inflammatory effects on colon tissue in an inflammatory state without affecting normal tissue,³⁸ and demonstrate chemoprevention effects on CRC.³⁹ In the human colorectal adenocarcinoma cell line DLD-1, the activation of NRF2-HO-1 signaling pathway induces CRC cell death through antioxidant stress, and may be related to the resistance of organobismuth (III) complex induced cell death.⁴⁰ The NRF2 inhibitor Brusatol causes rapid and transient depletion of NRF2 through a KEAP1 independent post transcriptional mechanism.⁴¹ Brusatol mediated NRF2 knockdown can inhibit the expression of HO-1 induced by organobismuth (III) complex and enhance its anticancer activity.⁴² Aldose reductase (AR) inhibitors have been confirmed to inhibit the growth of CRC cells in cultures and xenografts in nude mice.⁴² Inhibition of HO-1 activity can weaken the cytotoxicity against oxidative stress and reduce the efficacy of antioxidants.⁴³ Recent studies have shown that AR inhibitors increase the expression of NRF2 and related antioxidant enzymes in CRC cells, thereby increasing the expression of PGC-1 α , NRF1 and TFAM and reduces mitochondrial DNA damage, to regulate mitochondrial biogenesis and prevent CRC growth.⁴⁴

MicroRNA-222-3p (miR-222-3p) is also a key regulatory factor for oxidative stress,⁴⁵ increasing in the colon and circulating blood of CRC patients.^{46,47} Downregulation of miR-222-3p alleviates DSS induced oxidative damage by promoting BRG1 mediated NRF2 /HO-1 signaling in NCM460 cells, significantly alleviating oxidative stress and inflammation in the damaged colon of Ulcerative colitis (UC) and colitis-associated cancer (CAC) mice.⁴⁸ In addition, lactic acid bacteria and 5-aminosalicylic acid play a protective role in ulcerative colitis models by regulating gut microbiota and the NRF2/HO-1 pathway.⁴⁹

It is worth noting that during the growth and spread of cancer, there is potential crosstalk between NRF2 and the NF- κ B pathway. NF- κ B activation leads to NRF2 deficiency, resulting in increased cytokine production. Similarly, the activation of NRF2 also weakens NF- κ B and downstream signal transduction.⁵⁰ Targeted activation of the NRF2-HO-1 pathway can interfere with the NF- κ B signaling pathway, reduce NF- κ B mRNA expression in colon tissue, effectively reverse the deterioration of colon mucosal ulcers, and exhibit anticancer effects in experimental CRC induced Wistar rats^{51,52}.

NRF2 Promotes CRC Tumor Metastasis

The prognosis of CRC with or without distant metastasis varies greatly, with approximately 20% of CRC patients experiencing distant metastasis in clinical practice.^{53,54} Metastatic colorectal cancer is the main cause of patient mortality, with a 5-year survival rate of only about 14%.⁵⁵ Research has found that, the levels of KEAP1/NRF2 and SGP proteins, as well as their T/N (tumor/normal tissue) ratio, have varying degrees of correlation in CRC subjects with or without lymph node/distant metastasis.^{56,57} The T/N ratio of KEAP1 protein in the NRF2/KEAP1 pathway is an important predictor of lymphatic vessel infiltration (LVI) and can serve as a negative predictor of CRC metastasis.⁵⁶ Activate the KEAP1/NRF2 signaling pathway, NRF2 can directly induce the expression of miR-34a and miR-34b/c by occupying multiple ARE motifs in its promoter region, thereby inhibiting CRC metastasis.⁵⁸ In addition, as a key pathogenic factor and microbial biomarker for CRC, *Fusobacterium nucleatum* increases CYP2J2 and 12.13-EpOME transcription by activating the TLR4/KEAP1/NRF2 axis, and then promoting EMT and metastasis of CRC.⁵⁹

The activation of the NRF2/HO-1 pathway is also associated with increased invasiveness of CRC. For example, ethanol induces NRF2 and HO-1 nuclear translocation in response to oxidative stress and ER stress, endowing CRC with resistance to oxidative stress to maintain the survival of CRC cells, and inducing MMP2 and VEGF expression helps them achieve more aggressive phenotypes.⁶⁰ The promotion of cancer cell proliferation and metastasis in the tumor microenvironment (TME) by M2 tumor associated macrophages (M2-TAM) is a crucial step in the progression of CRC.⁶¹ The NRF2-HO-1 axis mediated antioxidant stress in CRC selectively survives CD163+M2-TAM in TME, leading to an increase in the frequency of M2-TAM infiltration.⁶² Previously, Consonni FM et al's research also showed that HO-1+CD163+M2-TAM, originating from F4/80HO-1 bone marrow precursor and dependent on NRF2 activation, promotes tumor angiogenesis and epithelial mesenchymal transition of tumor cells, and inhibits the function of CD8+T cells, leading to the formation of metastatic TME.⁶³ In addition, salicylic acid induces AMPK and inhibits c-MYC, transactivating the NRF2/ARE/miR-34a/b/c cascade to inhibit CRC cell migration, invasion, and metastasis⁶⁴.

NRF2 Participates in CRC Resistance

The resistance of cancer cells to treatment is the main cause of death in most cancer patients.⁶⁵ Chemotherapy based on oxaliplatin is the standard treatment for advanced CRC patients, and oxaliplatin resistance is a significant cause of death in CRC patients.⁶⁶ Research has found that,⁶⁷ oxaliplatin is an activator of the Nrf2 signaling pathway, which prevents the cytotoxicity of anticancer drugs by activating the KEAP1/NRF2 antioxidant system. Similarly, inhibiting NRF2 nuclear translocation can prevent KEAP1-NRF2 activation, thereby improving the resistance of human CRC cells to oxaliplatin.⁶⁸ In addition to oxaliplatin, multidrug resistance (MDR) is also a major obstacle to successful chemotherapy in CRC patients,⁶⁹ and one of its common forms is caused by the activation of the ABCB1 gene and its main product P-glycoprotein. Research has found that, NRF2 is a key transcription factor that regulates efflux transporters (including P-gp), There is a positive correlation between the protein levels of NRF2 and P-gp.⁷⁰ In CRC biopsy, the expression levels of NRF2 and ABCB1 (P-gp) were significantly increased at both mRNA and protein levels, while the expression levels of KEAP1 were obviously reduced in these samples.⁷¹

5-FU is the preferred chemotherapy drug for CRC, and its leading intestinal mucositis is the main limiting factor for anti-cancer treatment.⁷² Research has shown that CMP (Carboxymethylated pachyman, a polysaccharide extracted from traditional Chinese medicine *Poria cocos*) combined with 5-FU can regulate the NRF2-ARE signaling pathway to alleviate 5-FU induced colonic mucosal inflammation.⁷³ And another study shows that, Procyanidin B2 (PB2) reduces ROS accumulation through the NRF2/ARE pathway to protect the intestinal tract of mice from radiation induced damage.⁷⁴ Meanwhile, the NRF2/ARE pathway controls low-level ROS activation of β - catenin, thereby enhancing Wnt/ β - catenin signaling and promoting regeneration driven by leucine rich G-protein coupled receptor 5 positive intestinal stem cells (Lgr5+ISC), achieving similar results in models of intestinal organoid injury.⁷⁴ It is worth noting that, the activation of the NRF2 signaling pathway may not only be regulated by the ubiquitination of NRF2 controlled by KEAP1, but also by epigenetic modifications. Inhibiting methylation in the NRF2 promoter region can effectively activate NRF2, and the expression of NRF2 mRNA increases with a decrease in protein levels and enzyme activity of epigenetic modifying enzymes (such as DNA methyltransferase and histone deacetylase), and subsequently induces downstream antioxidant stress pathways⁷⁵.

In addition, the lncRNA MIR4435-2HG levels in cisplatin resistant cell line HCT116R were significantly elevated, and knocking out MIR4435-2HG significantly restored sensitivity to cisplatin.⁷⁶ Meanwhile, the mRNA levels of NRF2 and HO-1 are inhibited with the knockout of MIR4435-2HG, indicating that lncRNA MIR4435-2HG mediates cisplatin resistance through NRF2/HO-1.⁷⁶ Similarly, inhibiting the expression of NRF2/HO-1 pathway and pathway related biomarkers can reverse 5-FU resistance in colon cancer cells.⁷⁷

Other Regulation of NRF2 in CRC

In addition to participating in CRC resistance, metastasis, and early cell protective effects, NRF2 can also intervene in CRC progression by regulating metabolism, autophagy, and DNA damage. The ROS released by mitochondria activates NRF2, which affects CRC glycolysis, oxidative phosphorylation, mitochondrial biogenesis, and mitochondrial autophagy through the KEAP1/NRF2 pathway, and affects tumor metastasis.⁷⁸ ADSL expression prominently increases in CRC, indicating involvement in DNA synthesis DNA repair and upregulation of cell cycle genes accelerate cell cycle progression and significantly increase proliferation and migration of CRC cell lines. KEAP1-NRF2 is activated when ADSL is overexpressed, which is beneficial for the survival and proliferation of ROS accumulating cells, as well as DNA damage and tumorigenesis.⁷⁹ In addition, low levels of ITLN1 have been shown to be associated with obesity in the development of CRC and are independent prognostic factors for CRC.⁸⁰ ITLN1 inhibits tumor derived IL-17D mediated tumor neovascularization, bone marrow-derived EPC recruitment, and MDSC production and transportation through the KEAP1/NRF2/ROS/IL-17D signaling cascade dependent on PI3K/AKT/GSK3 β ,⁸¹ thereby benefiting CRC metastasis and immune suppression.

The Pleckstrin homology (PH) domain rich leucine repeat protein phosphatase 2 (PHLPP2) is a key regulator of cellular homeostasis and plays a tumor suppressive role in various human cancers.⁸² In CRC, PHLPP2 can inhibit the stemness of CRC cells by inhibiting the NRF2-ARE signaling pathway and increasing ROS levels.⁸³ In addition, sodium butyrate dose-dependently inhibits the growth of CRC HCT116 cells, at least partially due to inhibition of the NRF2 signaling pathway.⁸⁴ Sodium butyrate has the potential to demethylate the promoter of KEAP1 gene, leading to an increase in KEAP1 gene expression, thereby blocking NRF2-ARE signal transduction and NRF2 target gene expression, weakening the biological function of NRF2, and inducing CRC cell apoptosis by activating caspase to inhibit Bcl-xL protein.⁸⁵

The sustained activation of NRF2, which is beneficial to cancer cells, is known as “NRF2 addiction”⁸⁶ and is typically beneficial for CRC progression. Therefore, intervention targeting CRC cells with NRF2 addiction may become a new therapeutic target. Research shows that, L-selenocysteine can reduce NRF2 and autophagy pathway protein expression, selectively attacking NRF2 addicted cancer cells and ultimately leading to cell death.⁸⁷ It is worth mentioning that activating the KEAP1/NRF2 pathway to induce protective antioxidant stress response can effectively respond to the accumulation of ROS caused by radiotherapy and improve the effectiveness of radiotherapy.⁸⁸

The Regulation of NRF2 in CRC Ferroptosis

The Relationship Between NRF2 and CRC Ferroptosis: As a Major Negative Regulatory Factor

Ferroptosis is a newly discovered form of iron and reactive oxygen species dependent regulation of cell death, involving iron metabolism disorders and ROS accumulation in the plasma membrane. Ferroptosis is mainly induced by intracellular iron accumulation and lipid peroxidation. Excessive iron generates ROS through Fenton reaction to promote cell ferroptosis, which is accompanied by GSH depletion.

NRF2 is the key to cellular antioxidant response, widely involved in iron, lipid, amino acid, glucose metabolism, etc., and plays an extremely important role in the regulation of ferroptosis.⁶ Physiologically, the nuclear translocation of NRF2 activates the expression of genes under oxidative stress conditions, resulting in increased expression of antioxidant proteins, thereby reducing ROS and the LIP, to block the occurrence of ferroptosis (Figure 1). Therefore, NRF2 is the main negative regulator of ferroptosis, leading to abnormal inhibition of ferroptosis in tumors. Specifically, the downstream gene HO-1 of NRF2 has a dual role in ferroptosis, increasing LIP while producing antioxidants. It is worth noting that recent studies have gradually revealed the non-negligible role of ferroptosis in tumor suppression (Figure 1). Therefore, inducing ferroptosis in tumor cells by regulating NRF2 and related pathways has become a promising new method for treating cancer, including CRC.

NRF2 Regulates CRC Ferroptosis

Glutathione peroxidase 4 (GPX4) is one of the most important antioxidant enzymes, regulated by NRF2. GPX4, as a key regulatory factor of ferroptosis, has attracted considerable attention in fields such as cancer, cardiovascular, and

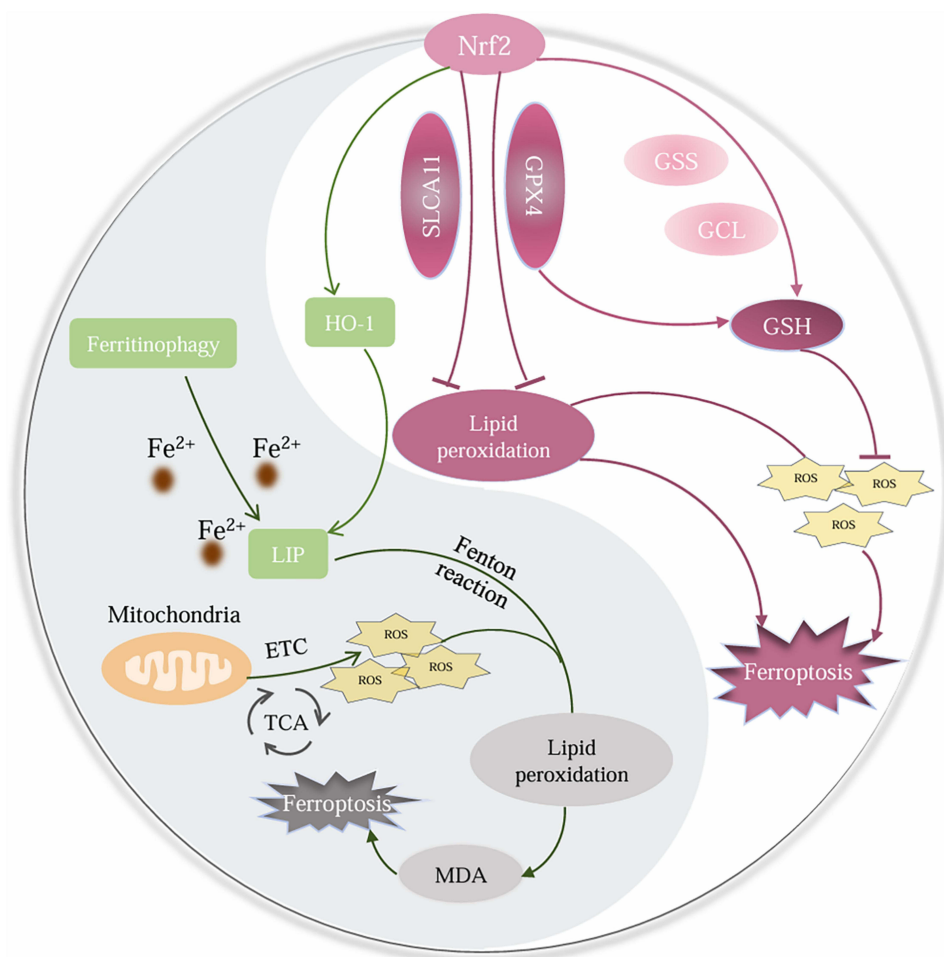


Figure 1 The Classic Mechanism of Ferroptosis and the Role of NRF2 in Regulating CRC Ferroptosis.

neuroscience in the past decade.⁸⁹ In preclinical studies, the regulatory mechanism of GPX4 level/activity has shown great potential for the treatment of ferroptosis related diseases. For example, upregulation of NRF2/GPX4 signaling pathway can affect the onset of pre-eclampsia by inhibiting ferroptosis,⁹⁰ and can save the cognitive dysfunction of diabetes encephalopathy.⁹¹ While inhibiting NRF2/GPX4 pathway to induce cell ferroptosis can accelerate female postmenopausal atherosclerosis,⁹² and can induce lung cancer cells and liver cells ferroptosis.^{93,94} In addition, inhibiting the NRF2/GPX4 pathway can enhance the sensitivity of multiple drug-resistant tumors to chemotherapy drugs and promote their ferroptosis.^{95–97} However, the significant impact of the NRF2/GPX4 signaling pathway in CRC has not been emphasized and summarized in previous articles. Based on this, we will summarize the role of the NRF2/GPX4 pathway in CRC ferroptosis.

Forkhead box transcription factor A2 (FOXA2) is a transcription factor that plays a crucial role in embryonic development, metabolic homeostasis and tumor cell proliferation. FOXA2 expression was significantly up-regulated in tumor samples of CRC patients, and was positively correlated with NRF2/GPX4 gene expression.⁹⁸ FOXA2 up-regulated indicating that the survival rate of CRC patients was poor.⁹⁸ In terms of mechanism, FOXA2 promotes CRC progression by inhibiting NRF2/GPX4 ferroptosis signaling pathway. Conversely, depletion of FOXA2 can weaken the activation of NRF2 pathway and reduce the level of GPX4 in CRC cells, thereby inducing ferroptosis.⁹⁸ Studies have shown that,⁹⁹ the expression of transferrin increased and the expression of GPX4 decreased in CRC cells treated with RSL3 (ferroptosis inducer). On the contrary, GPX4 overexpression attenuated RSL3 induced FCD. This suggests that decreasing GPX4 by inhibiting NRF2 may be the core factor of RSL3 induced ferroptosis in CRC cells.

The resistance of CRC to oxaliplatin is predictable, and the potential mechanism of its occurrence may involve inhibition of ferroptosis. An experiment targeting acquired oxaliplatin resistant CRC cells (HCT116 Or) as well as congenital resistant (H716) CRC cells revealed that inhibition of cell ferroptosis by the KIF20A/NUAK1/PP1 β /GPX4 pathway may be the basis of oxaliplatin resistance in CRC.¹⁰⁰ And inducing ferroptosis can significantly reverse oxaliplatin resistance in CRC cells.¹⁰⁰ It is worth noting that although apoptosis remains the main process of cell death in sensitized cancer cells in Caco-2 cell lines, GPX4/siRNA induced ferroptosis still kills cancer cells through alternative pathways to overcome oxaliplatin resistance.¹⁰¹

In addition to the NRF2/GPX4 signaling pathway regulating ferroptosis in CRC cells, other signaling pathways are also widely involved in the ferroptosis mechanism of CRC. Research has shown that activation of the PERK-NRF2-HO-1 signaling pathway mediated by ER stress can induce ferroptosis in CRC cells, accompanied by a decrease in GSH levels and an increase in lipid peroxidation.¹⁰² Under ER stress conditions, endoplasmic reticulum stress-related kinase (PERK) directly phosphorylates NRF2. Subsequently, phosphorylated NRF2 translocates to the nucleus and trans activates its effector HO-1 expression, leading to an increase in unstable iron pools and promoting lipid peroxidation, resulting in early ferroptosis in HCT116 cells.¹⁰² Furthermore, cetuximab enhances RSL3 induced ferroptosis by activating p38 MAPK and inhibiting NRF2/HO-1 axis.⁹⁹ It can be seen that inducing ferroptosis in CRC cells through the NRF2-HO-1 signaling pathway may expand the efficacy and scope of chemotherapy drugs. At the same time, ferroptosis can selectively target invasive cancer stem cells and has the potential to enhance the efficacy of immunotherapy, overcoming resistance to immunotherapy.¹⁰³

Sodium butyrate (NaB) can significantly increase RSL3 induced cell ferroptosis, and its sensitivity to ferroptosis is determined by FFAR2 -mTOR signaling transduction.¹⁰⁴ Specifically, NaB mediates the downregulation of SLC7A11 and GPX4 in a cAMP-PKA dependent manner through the FFAR2-AKT-NRF2 axis and FFAR2-mTORC1 axis, respectively, inducing lipid ROS production and promoting ferroptosis.¹⁰⁴ And ginsenoside Rh3 triggers CRC cell pyroptosis and ferroptosis through the Stat3/p53/NRF2 axis, with almost no damage to normal cells.¹⁰⁵ While uridine cytidine kinase like-1 (ucll1), which is highly expressed in a variety of cancers, has become a classical suppressor of CRC cell ferroptosis by stabilizing NRF2 and promoting SLC7A11 expression, playing an atypical role in inhibiting CRC cell ferroptosis¹⁰⁶.

In summary inducing ferroptosis in tumor cells has been widely studied as a promising new method for combating drug-resistant cancer. Inducing CRC ferroptosis through the NRF2 related signaling pathway may be beneficial for cancer patients by inhibiting oxaliplatin resistance, promoting tumor cell ferroptosis, and enhancing immunotherapy efficacy.

Targeted NRF2 in CRC Treatment and Prevention

The bidirectional regulatory role of NRF2 and related signaling pathways in CRC has been elaborated in detail in the previous section. On the one hand, the NRF2 signaling pathway can play a chemoprevention role in the occurrence of CRC, especially CAC, through anti-inflammatory and antioxidant mechanisms. Meanwhile, chronic toxin exposure, protein-protein interactions, epigenetic modification factors, transcription/translation regulatory factors, post-translational modifications, and mutations can also induce NRF2 in CRC for a long time and promote cancer occurrence, progression, metastasis, and chemotherapy resistance. More importantly, the activation and inhibition of the NRF2 signaling pathway may be beneficial for CRC in different microenvironments. Therefore, despite the considerable complexity and difficulty of targeting NRF2 in cancer, finding ways to manipulate each individual mode of NRF2 activation remains a key strategy for preventing or slowing down tumorigenesis, decreasing NRF2 addiction, cancer invasiveness and ultimately improving patient prognosis. Here, we summarize the NRF2 inhibitors and activators involved in CRC in recent years (including natural products, compounds, and some Chinese medicinal preparations) to serve as a reference for further research (Tables 1 and 2). Preclinical studies to intervene in CRC evolution by modulating NRF2 activity have been quite abundant, as we have summarized in detail in the table. Meanwhile, relevant clinical studies are gradually emerging. For example, the glutaminase inhibitor CB-839 in combination with 5-FU increased reactive oxygen species and induced nuclear translocation of NRF2, which in turn up-regulated the mRNA expression of uridine phosphorylase I, facilitated the conversion of 5-FU to its active compounds, and thus enhanced the inhibitory effect on thymidylate synthase and induced tumor regression in PIK3CA-mutant CRCs in multiple xenograft models.¹⁰⁷ And the combination of CB-839 and capecitabine, a prodrug of 5-FU, was demonstrated to be well tolerated with antitumor activity at biologically active doses in a subsequent Phase I clinical trial.¹⁰⁷ A prospective randomised controlled study experimentally showed that L-myostatin significantly elevated NRF2 levels (38.7%) and reduced the levels/activity of NF- κ B (27%) and TNF- α (36.6%), which was neuroprotective against oxaliplatin-induced peripheral neuropathy in colorectal cancer patients by targeting the NRF2 and NF- κ B pathways.¹⁰⁸ In contrast, the NEDD8-activating enzyme inhibitor Pevonedistat (TAK-924/MLN4924) exhibits clinical anti-tumor effects by non-specifically increasing NRF2 protein accumulation in patients with advanced solid tumours, including colorectal cancer.^{109,110} In addition, a novel synthetic triterpenoid and antioxidant inflammatory modulator, Bardoxolone methyl, effectively induced NRF2 and inhibited NF- κ B and JAK/STAT signaling pathway, and was able to activate NQO1, a key target of NRF2, in tumour tissues at the mRNA level, and clinical anticancer activity was observed in patients with solid tumors.¹¹¹

Summary and Future Directions

In conclusion, we summarized the opposite effects of Nrf2 and its related signaling pathways in the development and progression of CRC, which may be related to different stages of CRC and its heterogeneous tumor microenvironment. During the progression from adenoma to carcinoma, mutations occur in genes involved in nine driver signaling pathways, including the NRF2 signaling pathway.¹¹ In an immunohistochemical examination of 93 clinical tissues (65 carcinomas with corresponding surgical margins and 28 adenomas), it was found that NRF2 was progressively elevated in the direction of what appeared to be a normal mucosa-adenoma-carcinoma and showed significantly higher expression only in CRC with a rapid inflammatory peritumoral response, which may reveal a role for NRF2 in CRC carcinogenesis.¹² Furthermore, there was a significant difference between NRF2 expression and host immune response in adenoma specimens, with mean NRF2 expression correlating with high levels of dysplasia, indicating a poor response to treatment.¹² These studies demonstrate the complexity of the role of NRF2 in CRC, which is a double-edged sword. On the one hand, NRF2 resists malignant transformation of cells by maintaining the “redox balance” at normal levels, demonstrating a chemoprevention role. On the other hand, after malignant transformation, high NRF2 expression induces the transcription of antioxidants, giving them a pro-survival phenotype and ultimately the formation of chemo-resistant tumors.

The main body of chemoprevention effects of NRF2 appears in the inflammation-cancer sequence progression of CAC. Chronic inflammation strongly contributes to the onset and progression of CAC by persistently stimulating colonic epithelial cells (IECs) and inducing heterotypic proliferation through multiple mechanisms, including the induction of

Table 1 NRF2 Activators

Drug	Cell	Dose	Animal	Model	Medication Administration	Activation Method	Antitumor Mechanism	Cite
Cannabidiol	HCT116 p53wt HCT116; p53LS174T SW480	15μM; 20μM	SCID mice	HCT116 p53wt, HCT116 p53-/-*	20 mg/kg 1 p. q d 40 d	Promote Nrf2 nuclear translocation	Inducing apoptosis and macroautophagy	[112]
Allyl-isothiocyanate	HCT116	3, 10, 20, 30, 50, 100, 300pM	Male C57BL/6 mice	MC38*	1, 5mg/kg 1 p. q d 33 d	Increase Nrf2 Protein expression	Inducing Apoptosis	[113]
Salicylate	HCT116	5mM	NOD/SCID mice	SW620-Luc2*	SW620-Luc2 cells treated with salicylic acid 1 p. q d 5 w	Promote Nrf2 mRNA expression	Suppress metastasis Inducing cell apoptosis	[64]
Curcumin	HCT116	15μM	NOD/SCID mice	SW620-luc2*	4 × 10 ⁶ cells /0.2 mL iv		Suppress metastasis	[58]
Astragaloside IV	IEC-6	5, 20, 50pM	Male C57BL/6j mice	#	20, 40, 80mg/kg 1 g. q d 42 d	Promote Nrf2 mRNA expression	Reduce DNA damage	[114]
APE, AFE			C57BL/6 mice	#	150 mg/kg APE, 300 mg/kg APE 1o. q d 17 w	Promote Nrf2 expression	Chemoprevention	[115]
Piceatannol	NCM460	20, 80μM				Promote Nrf2 nuclear translocation	Reduce DNA damage	[116]
synthetic resveratrol-curcumin hybrid			Male Swiss mice, F344 male Wistar rat	****	8, 16, 32, 64mg/kg; 0.5, 1, 2mg/kg 1 g. bid 5 d	Promote Nrf2 mRNA expression	Chemoprevention	[117]
AF8c	HT29, HCT116	20μM	BALB/c nude mice	* HT29 Luc, HCT116 Luc	10, 20 mg/kg 1 p. q d 25 d	Promote Nrf2 nuclear translocation	Inducing Apoptosis	[118]
Tea-derived saponins myristicin	HEK293	5, 10, 15, 20, 25ug/mL	Male Sprague Dawley rat	***	150 mg/kg 1 g. q d 5 w	Promote Nrf2 mRNA expression Increase Nrf2 Protein expression	Inducing Antioxidant and Apoptotic Reversing the deterioration of colonic mucosal ulcers	[119] [52]
Huoxiang Zhengqi decoction			C57BL/6 mice	#	0.45, 1.35g/kg po. q d 6 w		Chemoprevention	[120]

(Continued)

Table 1 (Continued).

Drug	Cell	Dose	Animal	Model	Medication Administration	Activation Method	Antitumor Mechanism	Cite
Gurgem-7	Caco-2, CT26	50, 100, 250, 500, 1000, 2000µg/mL	male C57BL/6 mice	*	10, 20, 30, 50, 100mg/kg l g. q d 10 d	Promote Nrf2 mRNA expression	Promote intestinal injury repair	[121]
Procyanidin B2	NCM460, HCT116	20µM				Reduce Nrf2 degradation		[74]
Tagitinin C	HCT116	5, 10, 20µM	Sprague-Dawley rat	***	50 mg/kg po. q d 8 d	Activate Nrf2 nuclear translocation	Inducing ferroptosis	[102]
MMF						Increase Nrf2 expression	Anti- inflammatory and antioxidant	[122]
DHA	HCT116, NCI-H460	6.25, 12.5µM				Downregulate Keap1 Activate Nrf2 nuclear translocation	Inducing ferroptosis	[88]
Physodic acid, salazinic acid	DLD-1, HCT116	50µM	male BALB/c mice	#	0.25mg/mL drinking water containing 20 w	Increase Nrf2 gene transcription	Anti- inflammatory and antioxidant	[123]
Limonin						Promote Nrf2 mRNA expression		[124]
ShaoYao decoction	HT-29	2, 4, 6, 8mg/mL	FVB male mice	#	9.25, 18.5, 37g/kg drinking water containing 10 w	Promote Nrf2 mRNA expression	Prevention of oxidative damages	[125]
ShaoYao decoction			FVB male mice	#	9.25, 18.5, 37g/kg po. bid(2w) +tiw(8w) 10 w	Promote Nrf2 mRNA expression	Chemoprevention	[30]
CyCl	HCT116 HT29 SW620	50, 100µM				Activate Nrf2 nuclear translocation	Inducing Apoptosis	[126]
Sulforaphane(SFN)	HT-29 SW480	0, 10, 20µM				Inducing ERK phosphorylation to promote Nrf2 accumulation	Inhibiting cell proliferation	[127]
AG, HAG, PA	HCT-116	(100µg/mL (AG), 100µg/mL (HAG), (0.25µg/mL (PA)	C57BL/6 (WT), C57BL/6 Nrf2-/-	#####	75 mg/kg (AG), 75 mg/kg (HAG), 1 mg/kg (PA) po. q d	Activate Nrf2 nuclear translocation	Anti-inflammatory	[128]
Piperine			Male Wistar rats	####	30mg / kg po. fiw 18 w		Anti-inflammatory	[51]

Quercetin			Wistar rats	###	50 mg / kg. po. fiw 15/30w	Increase Nrf2 expression	Inhibition of DMA damage and cell proliferation	[32]
Igalan	HepG2	2.5, 5 μ M				Increase Nrf2 nuclear accumulation	Antioxidant	[129]
RSV, BHA			BALB/c mice, C57BL/6 Nrf2-/- mice	#	200mg/kg (BHA), 200mg/kg (RSV) I g. q d 30 d		Chemoprevention	[130]
RRx-001	HCT116 Caco2	0–10Mm; 5 μ g/ mL				Increase Nrf2 expression and nuclear accumulation	Promoting apoptosis	[131]
Luteolin	HT-29 SNU-407 FHC	0, 10, 30, 60 μ M				Promote promoter demethylation and increase Nrf2 transcription	Promoting apoptosis	[132]
Luteolin Luteolin	HCT116	5, 25 μ M	Male BALB/c mice	*#	1.2mg/kg P o. q d 17 w	Increase Nrf2 protein expression	Promoting apoptosis	[133] [31]
QYJD	HT29	5, 30 mg/mL	Male C57BL/6J mice	#####	1, 2, 4g/kg I g. tiw 5 w	Increase Nrf2 mRNA expression	Antioxidant	[134]
LUT	HCT116	7.5, 15, 30 μ M				Increase Nrf2 mRNA and proteins expression; Reducing CpG methylation in the Nrf2 gene promoter region	Chemoprevention	[75]
Ginnalin A	HCT116 SW480 SW620	24.8, 22.0, 39.7 μ M				Suppress Keap1, Promote Nrf2 nuclear translocation, Increase Nrf2 protein expression	Chemoprevention	[39]
fidarestat	SW-480 HT29 HCT116	10 μ M	nude mice	* HT29	50 mg/kg drinking water containing	Increase Nrf2 mRNA expression	Reduce DNA damage	[44]
cocoa			female BALB/c mice	#	50, 100 g/kg diet containing 62 d	Increase Nrf2 Protein expression and nuclear translocation	Chemoprevention	[34]
digitoflavone	Caco-2	1, 5, 10, 15 μ M	Male C57BL/6 mice	#	50 mg/kg I g. q d 12 w	Increase Nrf2 Protein expression and nuclear translocation	Chemoprevention	[36]

(Continued)

Table 1 (Continued).

Drug	Cell	Dose	Animal	Model	Medication Administration	Activation Method	Antitumor Mechanism	Cite
Nobiletin	RAW264.7 HCT116	0.5×, 1×, 2×	male CD-1 mice	#	500 ppm P o. q d 20 w	Increase Nrf2 protein expression	Inducing cell cycle arrest	[135]
Blanco, dried citrus peel	HCT116	1/2,000, 1/1,000, 1/500 (Dried Citrus Peel Extracts)	male F344 rats	**	1,000 ppm diet containing 4 w	Increase Nrf2 transcription	Reduce colonic crypt lesions	[136]
acetazolamide	Caco-2 SW48 HCT15	500μM	Male C57BL/6-ApcMin/+ mice		0.6, 1.2 mg diet containing 8 w	Activate Nrf2 transcriptional activity	Inhibiting proliferation and promoting apoptosis	[137]
Oxidized 5-ASA	HCT116	0.05, 0.25 mM	male Sprague-Dawley rats		500 μM, 400 μL P r. q d 6 d	Suppress Keap1, Increase Nrf2 nuclear accumulation		[38]
Melatonin			male Swiss Albino mice	##	1 mg/kg P o. b w 8 w	Increase Nrf2 protein expression	Reduce autophagy and slow down the CACC process	[138]
Genistein			Wistar rats	****	5 mg/kg P o. q d	Increase Nrf2 expression	Regulates tumor microenvironment	[139]
baicalein, negletein	HCT116	10, 20, 40μM; 50μM				Reduce Nrf2 phosphorylation and inhibit protein kinase		[140]
Brewers' rice			Male Sprague-Dawley rats	**	10% 20% 40% diet containing 20 w	Promote Nrf2 mRNA expression	Chemoprevention	[141]
Taxifolin			Male Swiss albino mice	****	4.0μg/kg P o. q d 15/30 w	Increase Nrf2 protein expression	Chemoprevention	[142]
Wogonin	Co-culture of HCT116 and THP-1	50μM	C57BL/6	#	60mg / kg I g. q d 111 d	Suppress Keap1, increase the stability of Nrf2 protein	Downregulate Keap1, increase the stability of Nrf2 protein, Inhibiting proliferation, Promote Nrf2 activation	[143]
Δ2-pioglitazone	HT29 HCT116	50μM				Promote Nrf2 nuclear translocation	Promoting apoptosis	[144]

Notes: *CRC xenograft mouse model, **AOM-induced Colorectal Aberrant Crypt, ***acetic acid induced UC model, ****DMH induced colon carcinogenesis model, #AOM/DSS induced CAC mouse model, #####DMH induced colon carcinogenesis Wistar rats, #####DSS-Induced Colitis Mouse Model, **AOM induced CAC mouse model, #DMH/DSS induced CAC mouse model.

Table 2 NRF2 Inhibitor

Drug	Cell	Dose	Animal	Model	Medication Administration	Inhibition Method	Antitumor Mechanism	Cite
RV-59	HCT116	1–5 μ M	Female BALB/c nude mice	shNrf2-HCT116*	2.5, 5mg /kg I p. q w 37 d		Suppress cytoplasmic Nrf2-mediated 5-fluorouracil resistance	[17]
SeC	WiDr C2BBe1	10, 50 μ M				Inhibit Nrf2 nuclear translocation Reduce Nrf2 mRNA stability	Inducing Apoptosis	[87]
Auraptene	CT26	50, 100, 200 μ M	Female BALB/c mice	* CT26	50, 100, 200 μ M. I p. q o d 14 d	Inhibit Nrf2 mRNA expression	Inducing Apoptosis	[145]
Fisetin	SW-480	30 μ M				Inhibit Nrf2 nuclear translocation	Inducing Apoptosis	[146]
2-HOBA			nude mice	## HCT116	1 mg.mL ⁻¹ drinking water containing 35 d	Inhibit Nrf2 nuclear translocation	Chemoprevention	[147]
			C57BL6	#	1 mg.mL ⁻¹ drinking water containing 56 d			
Quercetin	HCT116	10, 40 μ M				Inhibit Nrf2 expression	Inhibiting proliferation and inducing Apoptosis	[77]
Lysionotin	HCT116SW480	5, 15, 30 μ M	Male thymus free nude mice	* SW480	20mg / kg I p 24 d	Promote Nrf2 degradation	Inducing ferroptosis	[148]
quinacrine	HCT116 RKO	5 μ M	male BALB/c- nude mice	* HCT116	100 mg/kg QC+5 mg/kg 5-FU I p. t i w 36 d	Enhance Nrf2 degradation, Reduce the stability of Nrf2 protein	Enhancing 5-FU chemotherapy sensitivity	[149]
curcumin	HCT-8 HCT-8/5-Fu	10 μ M				Inhibit Nrf2 mRNA and protein expression levels	Reverse drug resistance, Inducing Apoptosis	[150]
Chemical structures of marine peroxy sesquiterpenoids	HCT116	25, 50, 100 μ M				Inhibit Nrf2 protein expression	Inducing Apoptosis	[85]
Metformin	HT29	10, 25, 50 mM				Inhibit Nrf2 transcriptional activation	Inducing Apoptosis	[151]
FMBP	HCT116DLD1	0.0125, 0.025, 0.0375, 0.05, 0.1 mg mL ⁻¹					Inducing ROS accumulation and cell death	[152]

(Continued)

Table 2 (Continued).

Drug	Cell	Dose	Animal	Model	Medication Administration	Inhibition Method	Antitumor Mechanism	Cite
Dihydromyricetin	HCT116/OXA HCT8/VCR	20, 50, 100, 200µM	Male BALB/ c mice	* HCT116/OXA	100 mg / kg (DMY), 100 mg / kg (DMY) +5 mg / kg (OXA) I p. b i w 4 w	Reduce Nrf2 transcription and nuclear translocation	Reverse drug resistance	[153]
Manuka honey	HCT-116 LoVo	5–15 mg/mL, 20–30 mg/mL	BALB/c nude mice	* HT29, HCT116	20mg/kg I g. q d 21 d	Reduce nuclear translocation	Inducing cell death by oxidative stress	[154]
Gasdermin D	HT29 HCT116	20, 40µM					Inducing pyroptosis and ferroptosis	[105]

Notes: *CRC xenograft mouse model, #AOM/DSS induced CAC mouse model, ###Subcutaneous transplantation tumor mouse model.

genetic changes, oxidative stress-driven DNA damage, aberrant immune responses, and disruption of intestinal flora.¹⁵⁵ The NRF2 pathway is involved in the maintenance of intestinal development and normal function, the development of UC, and UC-associated intestinal fibrosis and oncogenic effects, and mice deficient in NRF2 will have increased susceptibility to dextrose sodium sulfate-induced colitis and CRC.¹⁵⁶ In addition, inhibition of the signaling pathway of NRF2, a key transcription factor associated with cellular antioxidant responses and a central regulator in the maintenance of intracellular redox homeostasis, will disrupt the integrity of the intestinal barrier and induce colon tumorigenesis.⁴⁸ Therefore, activation of the NRF2 signaling pathway to alleviate oxidative stress in IEC can prevent and ameliorate UC and CAC occurrence. For example, in the context of oxidative damage in CAC mice, the activities of the antioxidant enzymes GSH-Px and SOD are significantly reduced, and ROS and MDA levels are significantly elevated in colonic tissues.⁴⁸ Increasing NRF2 expression increases the activity of the antioxidant enzyme SOD, decreases the level of MDA, and inhibits the levels of inflammatory markers NF- κ B, TNF- α , IL-1 β , and MPO in CAC mice, which has a preventive effect on the occurrence of CAC.⁴⁸ Activation of some classical pathways promotes the oncogenic effects of UC-CAC, and functional crosstalk between both NRF2 and NF- κ B was found.¹⁵⁷ By activating NRF2-mediated expression of phase II detoxification enzymes and reducing NF- κ B-associated inflammation could improve the intestinal environment, mucosal barrier, colonic and crypt disruption, and reduce ulceration and microbial translocation, which could reduce CAC occurrence.¹⁵⁸ In addition, regulation of NRF2 modulates various genes involved in cellular redox, protein degradation, DNA repair, xenobiotic metabolism, and apoptosis, again contributing to the prevention of CAC.¹⁵⁸ All these evidences reveal the promising application of regulating NRF2 activity in the prevention of CAC.

In addition to the classic chemo-preventive effect (anti-inflammatory and antioxidant), as well as the key role of Nrf2 in CRC progression, metastasis and drug resistance, we especially pay attention to the great potential of Nrf2 in CRC cell “erropptosis”. Notably, mismatch repair deficient and microsatellite instability high (D-MMR/MSI-H) represents a unique biomarker-defined group of cancers with higher tumor neoantigen loads and denser immune cell infiltration compared to mismatch repair proficient and microsatellite stable (p MMR/MSS) tumours, and is considered a positive immune checkpoint inhibitor (ICI) efficacy predictor. However, D-MMR/MSI-H-type CRCs account for only about 15% of all types of CRCs, and most MSS-type CRCs respond poorly to ICI therapy.¹⁵⁹ Studies have shown that the MSI score of CRC is negatively correlated with the NRF2 expression program,¹⁶⁰ revealing the negative role of NRF2 in anti-tumor immunity. TMB values correlate significantly with NRF2/KEAP1 mutations in nine cancer subtypes including CRC,¹⁶¹ and compared with unmutated cancers, cancers with mutations in PIK3CA, a gene associated with the NRF2 pathway, had a higher CRC had higher TMB.¹⁶² Furthermore, a subpopulation of NRF2 mutations in cancers is associated with poor prognosis but with increased PD-L1 expression, which may be beneficial for ICI therapy.¹⁶¹ However, although NRF2 mutations are associated with TMB-H, a negative correlation between NRF2 mutations and immune reactivity profile scores, negative ICI predictors, and CD8T cell infiltration may impair anti-tumor immune responses in MSS-type CRC.^{160,162} Therefore, modulating NRF2 expression in CRC and remodelling the CRC immune microenvironment may be an effective means to intervene in CRC progression.

In summary, for different CRC stages and tumor microenvironment, selective use of Nrf2 inhibitor activators will greatly improve the current situation and difficulties of CRC treatment. However, a large number of existing studies are still based on basic research at the cellular level and animal level, and further rigorous clinical research is still needed. We hope that through this review, researchers can see the broad prospects of NRF2 and its related signaling pathways in the application of CRC, and encourage more researchers to conduct more in-depth research to provide more solid and powerful evidence for the clinical application of NRF2 activators or inhibitors.

Disclosure

The authors report no conflicts of interest in this work.

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