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Review article

Pathological significance of heme oxygenase-1 as a potential tumor promoter in heme-induced colorectal carcinogenesis

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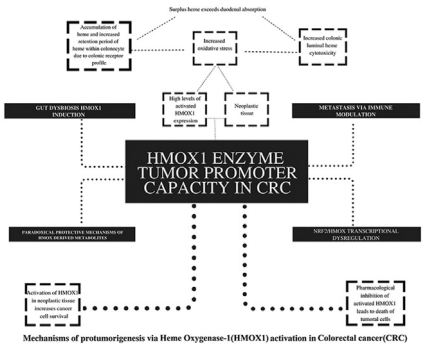
HIGHLIGHTS

- Heme Oxygenase-1 (HO-1) can behave as an oncogene beyond its beneficiary cytoprotective threshold.
- HO-1 exhibits paradoxical pro-tumor activity in colorectal cancer (CRC) via multiple mechanisms.
- Pro-tumoral effects of HO-1 induction are pertinent to advanced CRC.
- Pathways of HO-1 activation and downstream effects on tumor progression are potential anticancer targets.

GRAPHICAL ABSTRACT



Mechanisms of protumorigenesis via Heme Oxygenase-1(HMOX1) activation in Colorectal cancer(CRC)

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ABSTRACT

The significance of the heme-metabolizing enzyme heme oxygenase-1 (HMOX1) in the pathogenesis of colorectal cancer (CRC) has not been fully explored. HMOX1 cytoprotection is imperative to limit oxidative stress. However, its roles in preventing carcinogenesis in response to high levels of heme are not thoroughly understood. This study reviews various mechanisms associated with the paradoxical role of HMOX1, which is advantageous for tumor growth, refractoriness, and survival of cancer cells amid oxidative stress in heme-induced CRC. The alternate role of HMOX1 promotes cell proliferation and metastasis through immune modulation and angiogenesis. Inhibiting HMOX1 has been found to reverse tumor promotion. Thus, HMOX1 acts as a conditional tumor promoter in CRC pathogenesis.

Introduction

Colorectal cancer (CRC) is a significant cause of cancer-related morbidity across both sexes and is the second leading cause of cancer-related deaths globally.[1] Approximately 95% of patients with CRCs have adenocarcinomas that are categorized into three major subtypes: classical adenocarcinoma (CA), mucinous adenocarcinoma (MAC), and signet-ring cell adenocarcinoma (SRCC).[2] MAC and SRCC are less common and are associated with poorer prognosis and treatment outcomes.[3] The 2020 GLOBOCAN data predict that by 2040, CRC incidence will increase by 68% to 3.2 million new cases.[1,4] The trajectory of the estimated health burden is particularly concerning, with approximately 80% of new cases reported in countries with a high or very high human development index.[1] Therefore, there is an increasing focus on the role of

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environmental factors in the etiology of CRC. This involves an examination of the characteristics that constitute a westernized lifestyle, including obesity, a diet rich in processed foods and red meat, and low levels of physical activity.[5] Lifestyle factors have been heavily scrutinized because a large proportion of CRC cases are attributed to sporadic diseases characterized by spontaneous mutations in genes coordinating key cell cycle checkpoints and dysregulation of mismatch repair mechanisms.[6] A growing body of epidemiological meta-analytical evidence at the case-control and prospective cohort levels indicates an increased risk of CRC with higher dietary consumption of red meat.[7–12]

In recent years, there has been an accumulation of mechanistic evidence and proposals that attempt to explain the increased risk of cancer elicited by red meat consumption. These include genotoxic heterocyclic aromatic amines present in cooked meat and polycyclic aromatic hydrocarbons in smoked meat, the elevation of endogenously formed N-nitroso compounds, pro-oxidative effects of heme via cytotoxicity-related cell death, and lipid peroxidation.[12–15] The involvement of epigenetic factors, single nucleotide polymorphisms that dictate pro-carcinogenic behavior, and the role of the gut microbiome are developing fields in heme-induced CRC research.[10,12,16]

Heme iron is abundant in red meat and has received much scrutiny over the last decade for its pro-carcinogenic capacity.[17] Heme consists of a central iron atom within four nitrogen atoms of a protoporphyrin backbone. Heme is liberated by the digestion of the oxygen transporter proteins hemoglobin and myoglobin in the acidic gastric environment.[18] Physiologically, heme is processed in the duodenum by being internalized into epithelial cells by the heme transporter heme carrier protein 1 (HCP1). The heme oxygenase (HMOX) system catalyzes the oxidative degradation of heme.[18] However, a meal heavy in heme iron can override the physiological limits of the HMOX system in the proximal intestine, resulting in a greater proportion of heme reaching colonocytes, which have a reduced capacity to process heme.[13]

Multiple studies have demonstrated that large quantities of free heme iron induce oxidative stress and DNA damage, which increases the likelihood of the first phase of carcinogenesis: initiation.[19] Initiation involves gene mutations caused by cellular exposure to significant oxidative stress, resulting in the impairment of key biochemical signaling pathways.[20] The physiological function of heme oxygenase-1 (HMOX1) as a stress response enzyme is primarily to prevent initiation due to overwhelming levels of heme cytotoxicity. However, once the carcinogenic process has been established, HMOX1 appears to be involved in the promotion, progression, and metastasis of CRC. The extent of the cytoprotective capabilities of the HMOX system has not been well studied.

This study aimed to review the significance of HMOX1 in promoting and sustaining heme-induced carcinogenesis, its role as a 'cytoprotective enzyme,' and its potential role as a tumor promoter.

Heme oxygenase-1 activity in physiological and stressful cellular environments

Physiological cytoprotective role of intestinal heme oxygenases

HMOXs are microsomal heme-related enzymes involved in the initial rate-limiting step of heme catabolism. HMOXs reside within the endoplasmic reticulum and exist in three isoforms: HMOX1, HMOX2, and HMOX3.[20] Most of the available data on the HMOX suite relate to HMOX1. HMOX1 is an inducible form of the HMOX system that acts as a protective response to maintain redox homeostasis and prevent carcinogenesis in healthy cells when exposed to stressful environmental stimuli, including elevated levels of heme substrates, reactive oxygen species (ROS), UV radiation, and hypoxia.[6] The physiological significances of HMOX2 and HMOX3 are not well understood. HMOX3 is considered a pseudogene that does not encode a protein with functional heme degradative capacity, despite sharing 90% homology with HMOX2.[21,22] HMOX2 is involved in heme catabolism under conditions of normal physiological intake.[23] The maladaptive response of HMOX2 to cellular oxidative stress was demonstrated in a recent study by Gamage et al. that found significant downregulation of HMOX2 in normal colonic epithelial cells (fetal human cells [FHC]) at hemin concentrations $\geq$25 μmol/L.[24] The limited functionality of HMOX2, despite sharing 40% amino acid homology with HMOX1, may be due to differences in its transcriptional control. Multiple stress-related transcription factors are involved in modulating HMOX1 activity, including Jun B proto-oncogene, activator proteins 1 and 2 (AP-1 and AP-2), nuclear factor kappa B (NF-kB), hypoxia-inducible factor (HIF-1), and nuclear factor erythroid 2-related factor 2 (Nrf2), which bind to corresponding regulatory elements in the promoter region of the HMOX1 gene.[21,25] Unlike HMOX1, HMOX2 is constitutively expressed and transcriptionally induced via a single functional response element in the promoter region, known as the glucocorticoid response element.[25] Higher levels of HMOX2 expression have been noted in CRC and adjacent non-neoplastic tissues, suggesting the deregulation of physiological homeostatic mechanisms.[24] It is unclear whether cell-specific expression of HMOX2 compensates for reduced expression of HMOX1 in CRC; however, it can be hypothesized that compensation is unlikely to be due to activation via different stimuli and transcriptional pathways.

Heme metabolism by HMOX1 produces equimolar quantities of Fe2+, carbon monoxide (CO), and biliverdin.[18] These byproducts contribute to the multidimensional cytoprotective effects of HMOX1. Fe2+ stimulates the synthesis of ferritin heavy chains (FtH) and induces expression of genes for iron efflux pumps, thus attenuating oxidative stress and preventing Fenton redox hydroxyl radical formation.[19,26] Seiwert et al. reported that hemin concentrations $\geq$20 μmol/L were associated with substantial ROS formation in a concentration-dependent manner in human colonic epithelial cells.[19] Genotoxic outcomes of these ROS, including destabilization of double bonds in DNA and oxidative DNA damage in primary colonocytes, have been observed at $\geq$100 μmol/L of hemin.[13,27] These heme concentrations are comparable to the luminal heme concentrations found in humans after red meat consumption.[19] Mutations in FtH result in the long-term mucosal retention of free Fe2+.[28] Thus, inadequate adaptive upregulation under highly oxidative cellular conditions increases the production of hydroxyl radicals. Interestingly, upregulation of FtH in some CRC specimens has been noted, reducing oxidative stress and conferring cytoprotection to tumor cells.[23] Taken together, ferritin synthesis via HMOX1 induction is likely to be cytoprotective in pre-cancerous conditions and confers an enhanced survival advantage on transformed cells.

HMOX1 is upregulated in response to oxidative stress to prevent heme-mediated cellular damage, suggesting its role as a stress protein.[29] These unfavorable cellular stress environments are created when heme levels surpass normal physiological intake.[13] Up to 90% of the total consumed heme may reach the colon.[26,27] Increased transit time in the colon allows for more significant interactions with the colonic mucosa.[30] Thus, the inherent biological limitation of the proximal intestine in absorbing most of the consumed heme, particularly from heme-rich diets, would favorably induce HMOX1 activation in the colon.

Excessive heme saturates the available ferritin and the capacity of HMOX1, resulting in excess free Fe2+ accumulation and superoxide anion radical formation, a critical component in the initiation of carcinogenesis.[31] A recent study demonstrated that free heme iron might have a greater role in inducing DNA strand breaks in human colonocytes than the inorganic iron byproducts of heme metabolism.[19] Biliverdin and its metabolite bilirubin reduce lipid membrane damage and lipoprotein injury by scavenging peroxyl free radicals, thereby inhibiting lipid peroxidation and suppressing cellular toxicity.[32,33] CO is cytoprotective through its anti-apoptotic effects and by inhibiting unregulated cell proliferation by acting through the mitogen-activated protein kinase and p38β pathways.[18,21] In addition, CO increases tissue perfusion and has a pro-angiogenic capacity.[34] Thus, HMOX1 is cytoprotective via the metabolites produced during heme degradation.

Limited extent of heme oxygenase-1 cytoprotection at higher heme exposures

The importance of HMOX1 in heme detoxification and its protective effect on healthy colonic cells amidst mildly elevated heme levels beyond normal physiological concentrations have been widely reported in the literature.[19] The cytoprotective activity of HMOX1 has been extensively reviewed in the literature, with recent studies on HMOX1 knockout mice (*Hmox1*−/−) demonstrating the impact of cellular toxicity in the absence of the enzyme.[35] However, studies on the extent of cytoprotection mediated by HMOX1 following exposure to excessive heme concentrations are limited. A recent study has demonstrated that HMOX1 functions solely as a cytoprotective agent against heme detoxification.[35] This conclusion was derived from the observation of the genotoxic effects of heme in the absence of activated HMOX1.

Contrary to the general understanding that HMOX1 plays an absolute role in cytoprotection, some studies have suggested that a beneficial threshold exists for HMOX1 to prevent damage from heme accumulation.[36] A few studies have noted that cytoprotective effects exist at a certain level of HMOX1 expression, that is, approximately a two to five-fold increase from basal HMOX1 expression.[33,36] A roughly 3-fold increase in HMOX1 expression was observed, with a 40% reduction in heme levels. Further increases in HMOX1 expression were associated with increased heme content accompanied by cellular dysfunction. An excessive 15-fold increase in HMOX1 expression results in lipid peroxidation and oxidative cell injury.[36] This is supported by a recent study that found HMOX1 expression higher in colon cancer cells (SW480) than in normal colonic epithelial cells (FHC) at all hemin concentrations (25–250 μmol/L).[24] More importantly, HMOX1 expression is significantly higher in adjacent non-neoplastic tissues than in CRC tissues.[24] Interestingly, downregulation of HMOX1 occurred in normal colonic epithelial cells at the highest hemin concentration (250 μmol/L),[24] possibly suggesting the effectiveness of HMOX1 as a cytoprotective enzyme that works within a specific range of expression. Hence, it seems a beneficial threshold exists at which HMOX1 provides cytoprotection to prevent neoplastic changes, but beyond its upper limit, HMOX1 may behave as an oncogene that facilitates tumor progression.

Increasing intracellular heme and excess luminal heme at the apical membrane stimulates the activation of antioxidant signaling pathways, such as HMOX1 upregulation.[37] However, the resulting upregulation of HMOX1 may be inadequate owing to the overwhelming luminal cytotoxicity and resulting oxidative injury to the colon epithelia. Thus, the HMOX1 cytoprotective effect may be conditional and depend on the extent of the oxidative agent burden that the cellular environment can tolerate. It would be of interest for future studies to define the cytoprotective limits of HMOX1 by quantifying HMOX1 expression levels. These levels may have potential significance as a diagnostic marker in biopsies of inflamed colonic tissue and thus curtail a preneoplastic lesion from proceeding into carcinogenesis.

Involvement of heme oxygenase-1 in heme-induced carcinogenesis

Heme-induced cytotoxicity can occur through multiple pathways, including the extreme hydrophobicity of cytotoxic heme factors, which induces lipid peroxidation, generation of ROS via heme metabolism, formation of N-nitroso compounds, and alteration of the intestinal Gram-positive bacterial population, eventually leading to dysbiosis, all of which can contribute to the initiation of CRC.[6,31] However, only a few studies have investigated the effects of HMOX1 on heme-induced carcinogenesis.

Heme oxygenase pathways in colorectal cancer carcinogenesis

The alternate role of heme oxygenase-1 as a potential tumor promoter in colorectal cancer

Tumor promoters facilitate the progression of existing cancer processes via factors that provide growth advantages.[38,39] Tumor promoters are characterized by their ability to inhibit the apoptosis of abnormal cells marked by genotoxic lesions and to promote the clonal proliferation of preneoplastic cells and metastatic spread. In addition, inhibition or knockout of the tumor promoter should prevent further advancement of tumorigenesis and may increase the susceptibility of the tumor to apoptosis due to the weakening of its defense systems.[39,40]

Excess heme induces cytotoxicity in the colonic epithelium, highlighting its ability to behave as a carcinogen.[41] This results in cell transformation. Growing evidence of HMOX1 in these initiated environments demonstrates its influence on tumor growth and expansion, including cell proliferation, metastasis, angiogenesis, and inhibition of apoptosis of cancerous cells, suggesting an alternate conditional role for HMOX1 as a potential tumor promoter [Figure. 1].

Advantage of pleiotropic metabolites in tumorigenesis

As introduced earlier, induction of HMOX1 provides pleiotropic protection through its metabolites. HMOX1-derived CO has widely known anti-inflammatory properties, mediated primarily by acting on the intracellular target p38 mitogen-activated protein kinases (MAPK).[21] Downstream effects include inhibiting the release of pro-apoptotic factors due to mitochondrial membrane permeabilization, stabilizing the anti-inflammatory cellular environment, and inducing the expression of protective cytokines against inflammation-induced apoptosis.[21] Although the HMOX/CO axis appears to be a promising therapeutic target, multiple reports have shown that CO plays a dual role in tumor pathophysiology.[6,21,42,43] CO has been confirmed as a diagnostic marker of CRC because of the direct relationship between increased HMOX1 expression and CO circulation.[44] Increased CO levels in CRC have been shown to exert a paradoxical pro-tumor role via mechanisms that may involve extending anti-inflammatory properties to carcinogenic tissue, thus maintaining abnormal cell proliferation pathways that promote the progression of tumorigenesis.[6] Furthermore, the activation of the phosphoinositide 3-kinase (PI3K)/ protein kinase B (AKT) pathway via the HMOX/CO axis prolongs the survival of CRC cells by inhibiting pro-apoptotic signals that induce cell cycle arrest.[21] Moreover, increased CO levels promote tumor growth and metastasis via immunological escape by modulating the immune surveillance system.[6,21]

In contrast, CO may also exert an antitumor effect in CRC. Studies have shown that treatment of human CRC cells and HCT116 colon carcinoma cells with carbon monoxide-releasing molecule 3 (CORM-3) induces apoptosis via the cleavage of pro-apoptotic proteins, caspase-3 and poly(ADP)ribose polymerase (PARP).[21] Interestingly, exogenous CO resulted in decreased phosphorylation of AKT, thus increasing the cell susceptibility to cell-mediated apoptotic death.[45] Exogenous CO appears to introduce an imbalance in the cellular energetics of the tumor environment, which may be maintained by an optimal concentration of endogenously produced CO, inducing mitochondrial stress, and consequently, cell death.[21,42] Thus, endogenous CO from HMOX1 metabolic activity offers a selective advantage for the progression of tumorigenesis and is also a promising target in the treatment of CRC.

Significance of the nuclear factor erythroid 2-related factor 2/heme oxygenase and BTB and CNC homolog 1/heme oxygenase transcriptional axes to tumor progression

Several key stress-responsive transcription factors are involved in regulating the transcription of HMOX1, including BTB domain and CNC homolog 1 (BACH1), hypoxia-inducible factor 1 alpha (HIF-1α), Nf-kB, and Nrf2.[46,47] Overactivation of certain transcription factors, in particular Nrf2, has been implicated to have a significant role in colorectal carcinogenesis.[6]

Nrf2 is a leucine zipper transcription factor that controls the expression of multiple cellular defense genes, including *HMOX1*.[47] Under basal conditions, Nrf2 is continuously inhibited from binding to the antioxidant response element (ARE) on *HMOX1*. This is mediated by its

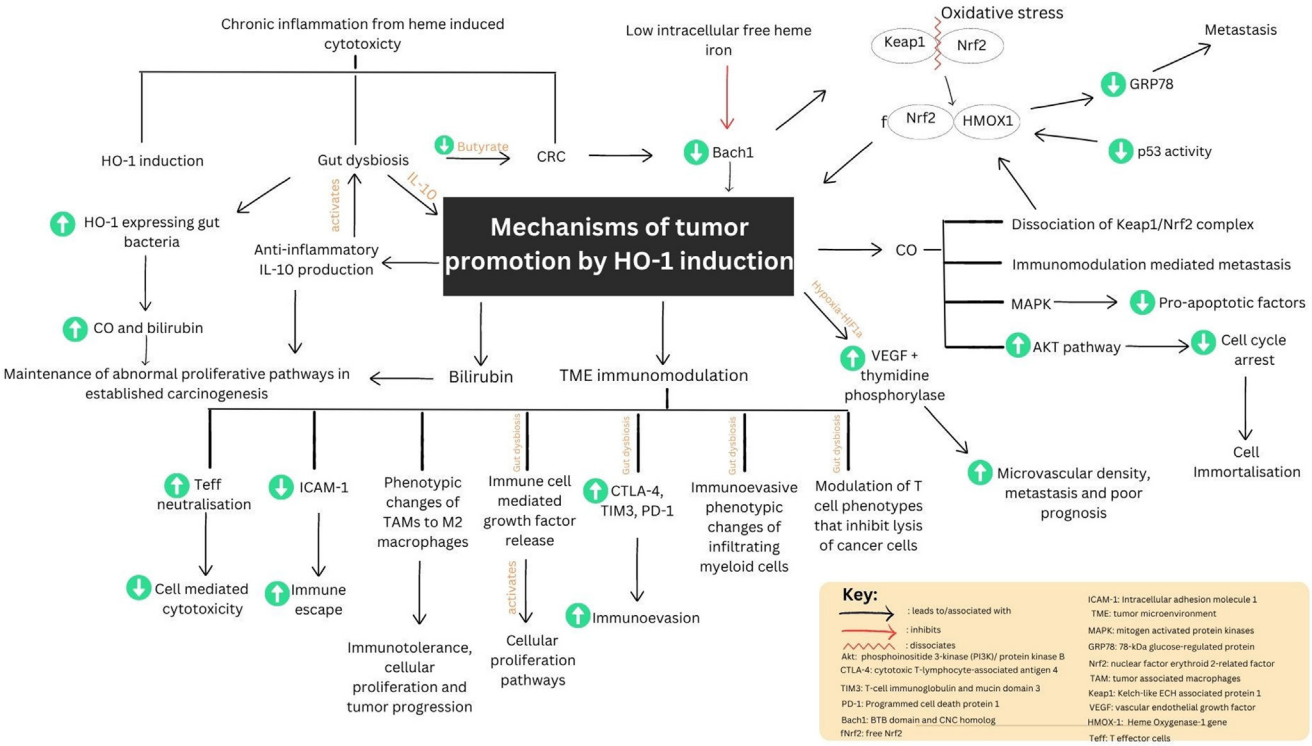

Figure 1. HMOX1 overactivation in CRC induced by multiple mechanisms including chronic inflammation arising from prolonged heme cytotoxicity, gut dysbiosis, low BACH1 levels, and high levels of free Nrf2 transcription factors. Low BACH1 levels augment dissociation of the Keap1/Nrf2 complex. Low p53 activity increases Nrf2/*HMOX1* stimulation. High free Nrf2 levels lead to inappropriate HMOX1 activation and increase the risk of metastasis via decreased GRP78. Chronic inflammation promotes pro-colitic and carcinogenic pathways via gut dysbiosis-induced HMOX1. HMOX1-derived metabolites CO and bilirubin maintain abnormal proliferative pathways in established carcinogenesis. CO also promotes cell immortalization, immunomodulation-mediated metastasis, and increased dissociation of the Keap1/Nrf2 complex. HMOX1 induction in neoplastic conditions leads to TME immunomodulation. This involves modulation of T cell phenotypes that inhibit the lysis of cancerous cells, immune-evasive phenotypic changes of infiltrating myeloid cells, and modulation of circulating macrophages in the TME. These changes promote immunotolerance, cellular proliferation, and tumor progression. HMOX1 induction via the hypoxia-HIF1a transcription pathway leads to increased expression of VEGF and thymidine phosphorylase, key mediators of increased microvascular density and metastasis. BACH1: BTB domain and CNC homolog 1; CO: Carbon monoxide; CRC: Colorectal cancer; GRP78: 78-kDa Glucose-regulated protein; HIF1a: Hypoxia-inducible factor 1 alpha; HMOX: Heme oxygenase; Keap1: Kelch-like ECH-associated protein 1; Nrf2: Nuclear factor erythroid 2-related factor 2; TME: Tumor microenvironment; VEGF: Vascular endothelial growth factor.

association with the cytoplasmic repressor, Kelch-like enoyl-CoA hydratase (ECH)-associated protein 1 (Keap1), which is involved in Nrf2 degradation via ubiquitination, followed by proteasomal inactivation.[6] Oxidative stress disrupts the Keap1/Nrf2 complex. Subsequent nuclear translocation of the released Nrf2 leads to it binding to ARE in the promoter region of *HMOX1*, inducing HO-1 activation.[46] Nrf2 hyperactivation due to loss-of-function mutations in Keap1 is a common finding in several cancers, including CRC. In addition, epigenetic changes involving demethylation of the Nrf2 promoter and hypermethylation of Keap1 stimulate HMOX1 overactivation.[48] Dysregulation of the Nrf2/HMOX axis, as demonstrated by these somatic mutations and epigenetic alterations, reduces Keap1-mediated and proteasomal degradation of Nrf2, culminating in overstimulated HMOX1.[49] This leads to inappropriately amplified ROS detoxification capabilities in neoplastic tissues, improving cancer cell survival, and increasing cancer therapy resistance.[6,49] Overexpression of HMOX1 may also initiate a positive feedback loop that augments Nrf2 signaling owing to increased susceptibility of Keap1/Nrf2 dissociation at higher levels of CO.[46,47] Thus, dysfunction of the Nrf2/HMOX axis alters HMOX1 behavior, akin to a tumor promoter that creates a hostile microenvironment conducive to tumor growth and progression.

Transcription factor BACH1 may also play a role in CRC carcinogenesis. BACH1 represses the transcription of HMOX1 in low-heme environments. This reduces the metabolic activity of HMOX1, allowing free heme iron to accumulate.[47,50] Free heme iron is more cytotoxic than the inorganic iron produced by heme metabolism.[19] Gamage et al. have also confirmed that low hemin concentrations (25 μmol/L) were sufficient to induce oxidative stress in normal colonic epithelial cells.[24] BACH1 binds more competitively to the ARE of *HMOX1* than Nrf2 under low cellular oxidative stress conditions, thereby inhibiting HMOX1 activation.[47] However, increasing heme levels stimulate a conformational change in BACH1 that negatively affects its ability to bind to the promoter region of *HMOX1*.[47,50] This allows Nrf2 to bind freely to HMOX1 and upregulate its cytoprotective response against overwhelming oxidative stress.[47,50]

Furthermore, Jiang et al. found low levels of BACH1 expression in CRC, which may be due to high levels of oxidative stress, thus promoting the unrestrained access of Nrf2 to HMOX1.[51] Therefore, BACH1 may play a significant role in the initial stages of carcinogenesis owing to its repressive effect on HMOX1 in low-heme environments. However, it is difficult to ascertain the effect of the BACH1/HMOX1 axis on CRC development because of the limited number of studies available. Thus, future studies should determine whether chronic levels of low heme increase the susceptibility of normal colonic epithelia to the early stages of carcinogenesis.

Gut microbiota and heme oxygenase-1 induction

Chronic intestinal inflammation is strongly associated with gut dysbiosis.[52] Prolonged inflammation can initiate changes in the composition of the gut microbiota, which can influence cell transformation and tumor progression.[16] Constante et al. found that mice fed heme showed significant alterations in the makeup of their microbiota, favoring the growth of *Enterobacteriaceae*, which are known to influence the

bioactivation of carcinogens.[52] Microbiome sequencing has shown that *Enterococcus, Escherichia coli, Shigella,* and *Klebsiella* species are significantly more abundant in the gut microbiota of CRC tissue compared to normal colon tissue of healthy controls.[16] In addition, the heme-induced dysbiotic signature is further characterized by reduced protective gut commensal bacteria and the growth of heme-metabolizing bacteria, such as *Bacteroides fragilis.*[53] These profound shifts in microbiota composition create a pro-inflammatory environment that facilitates colitic and pro-carcinogenic host responses.[54] Reduced levels of the beneficial gut bacteria *Firmicutes* lead to a reduction in the production of butyrate, a bacterial byproduct with anti-inflammatory and antitumorigenic properties.[52] A reduced butyrate level due to an imbalance within the gut microbiota community has been implicated as a potential mechanism for aggravating colitis and promoting adenoma formation.[16] Dysbiosis due to intestinal inflammation stimulates HMOX1 induction. Some bacteria exhibit heme uptake and metabolic machinery analogous to HMOX1.[55] Recent developments have shown that some *E. coli* strains express an HMOX1 analog capable of producing significant levels of CO.[16,43]

HMOX1-derived metabolites appear to have double-edged roles in their influence on the gut microbiome. CO can increase the clearance of some bacteria such as *E. coli, Enterococcus faecalis, Salmonella typhimurium,* and augment HMOX1-induced phagocytic immune action. However, bilirubin, a downstream metabolite of HMOX1, suppressed this bactericidal effect.[55] Impaired clearance of *Enterobacteriaceae* may allow the accumulation of toxic effects on colonic epithelia, significantly contributing to carcinogenesis.[53] In addition, HMOX1 induction by the microbiota is mediated by interleukin (IL)-10. Germ-free mice colonized with normal gut microbiota under conditions in which IL-10 was inhibited did not induce HMOX1 expression compared to the control. Upregulated HMOX1 further augments the response to IL-10, accentuating the anti-inflammatory action of IL-10 by downregulating pro-inflammatory cytokine production.[43] Although this may reduce inflammation in healthy colonic epithelia, the anti-inflammatory action mediated by gut bacteria-induced HMOX1 may promote carcinogenesis. Gut dysbiosis is a developing field in cancer research and further studies are needed to determine the influence of the gut microbiome and HMOX1 induction on carcinogenesis.

Pro-angiogenic capacity of heme oxygenase-1

High levels of HMOX1 expression have been associated with pro-angiogenic activity in various tumors, both *in vitro* and *in vivo*. A pro-angiogenic environment brought about by HMOX1 induction is evident in CRC.[56,57] Hypoxia in the tumor microenvironment stimulates HMOX1 activation.[48] HIF-1α is a transcription factor that regulates the expression of genes responsive to hypoxia, including *HMOX1*.[42] The HMOX/CO axis appears to drive blood vessel formation by inducing the expression of pro-angiogenic mediators, such as vascular endothelial growth factor (VEGF). This binds to VEGF receptors on endothelial cells, stimulating angiogenesis.[48] Overexpression of HMOX1 has been correlated with the release of a pro-angiogenic enzyme, thymidine phosphorylase, which in addition to VEGF, contributes to increased microvascular density, the metastatic spread of cancer, and poor survival outcome in CRC.[29,56,57] Furthermore, activation of the Nrf2/HMOX1 axis facilitates the migration and invasiveness of colon cancer cells by downregulating the 78-kDa glucose-regulated protein (GRP78), an important endoplasmic reticulum chaperone protein involved in preventing the export of misfolded proteins.[48]

Moreover, HMOX1 expression in macrophages in the CRC tumor microenvironment is polarized toward a pro-angiogenic phenotype.[48,58] Thus, the interacting suite of HMOX1-induced macrophages found in the CRC tumor microenvironment may also contribute to the invasiveness and angiogenic potential of tumors. Finally, HMOX1 inhibition results in decreased VEGF release and tumor angiogenesis, as demonstrated in an HCT-15-induced xenograft model of CRC.[48] Thus, HMOX1 induction in CRC may promote tumor progression via mechanisms that induce angiogenesis. However, there is variability in literature reports on HMOX1 induction and angiogenesis across different tumor types. Future studies should investigate the reasons for these differences, as signaling systems specific to the CRC tumor microenvironment may influence HMOX1 activity.

Heme oxygenase pathways in modulating the colorectal cancer tumor microenvironment

A growing body of evidence suggests that HMOX1 can modulate the tumor microenvironment in colon cancer cells via mechanisms of immunological escape and interference with effector T cell (Teff) recruitment, thus promoting cancer progression.[48,59]

High tumor infiltration by Teffs increases the cytotoxicity of tumor cells via direct or antibody-dependent mechanisms. This is in contrast to regulatory T cells (Treg), which promote tumor survival by neutralizing Teff cells and thus weaken the immune system's cell-mediated cytotoxic responses against CRC.[29,59,60] The effect of HMOX1 activity on anti-cancer immunity is regulated by the interplay between intracellular adhesion molecule 1 (ICAM-1) and suites of T cells, including Th1 and CD8+.[59] ICAM-1 is an adhesion molecule necessary for the trans-migration of immune cells. This is assisted by a concentration gradient established by the chemokine CXCL10, which allows the infiltration of Teff cells into the tumor microenvironment. Reduced ICAM-1 expression causes CRC cells to become less recognizable by the immune system. Impaired secretion of CXCL10 reduces the probability of infiltration and cytotoxicity-mediated damage, allowing CRC cells to escape immunosurveillance.[59]

HMOX1 overexpression in macrophages, dendritic cells, and immunocytes has also been observed in stromal cell populations within the tumor microenvironment, such as macrophages, dendritic cells, and immunocytes.[43] Various mechanisms for HMOX1 induction in infiltrating monocytes have been described. These involve the influence of cytokines IL-6 or IL-10 produced by cancer-associated fibroblasts, endothelial cells, tumor-associated macrophages (TAMs), or other signaling pathways, including activation of HIF-1α via tissue hypoxia and metabolic activity of the tumor microenvironment.[61] The induction of HMOX1 polarizes the phenotypic expression of these TAMs to M2 macrophages, which are anti-inflammatory and reduce oxidative stress in the tumor microenvironment.[43,60] This results in immunotolerance and promotes cell proliferation, tumor progression, and angiogenesis.[48] In addition, HMOX1 overactivation in TAMs has been shown to augment their immune escape capabilities.[48]

Furthermore, HMOX1 induction in myeloid-derived suppressor cells weakens T-cell cytotoxicity via activation of the NF-kB/signal transducer and activator of transcription 3 (STAT3) signaling pathway. This promotes cell immortalization, development of a pre-metastatic niche, and increased resistance to immunotherapy.[58] Recent evidence demonstrates that HMOX1-induced modulation of immune cell communication with the CRC tumor microenvironment is positively associated with lymph node metastasis and poor survival outcomes in advanced stages.[48,62] Therefore, HMOX1 induction in the tumor microenvironment facilitates tumor progression through immune evasion and increases chemoresistance.

Gut dysbiosis due to chronic inflammation can facilitate CRC metastasis by modulating immune cell activity in the tumor microenvironment.[16] Changes in the intestinal microbiota of patients with CRC have been associated with a pro-inflammatory cytokine environment.[53] These include TNF-α, IL-1, IL-6, IL-8, IL-17, IL-23, and IL-β, all of which have been implicated in roles contributing to intestinal tumorigenesis.[63] Chronic activation of these inflammatory signals promotes immunosuppression and thus tumor progression. This is mediated by stimulation of infiltrating monocytic cells, inducing the release of growth factors that act on the MAPK, wingless-type (Wnt)/β-catenin, or PI3K/AKT/Mammalian Target Of Rapamycin (mTOR) signaling pathways.[53,63] In addition, dysbiosis may disrupt inflammasome activity and thus play a role in the immune-evasive phenotypic changes of tumor-invading myeloid cells

such as TAMs.[43,63] This is supported by studies demonstrating that bacteria specific to the CRC gut microbiome promote tumor progression through the induction of TAMs.[64,65] In addition, there is variability in the extent of pro-tumorigenicity of specific immune cell phenotypes at different stages of CRC. For example, the tumor-associated neutrophil phenotype plays a more pronounced pro-tumor role in established tumors. Furthermore, gut dysbiosis can reprogram adaptive antitumor responses, thereby increasing cancer cell longevity and immune escape.[63] This is achieved through the modulation of T cell phenotypes, which decreases the susceptibility of cancer cells to lysis by inhibiting cytotoxic CD8+ T cell activation.[42,63] Moreover, microbiota induces the expression of inhibitory molecules such as cytotoxic T lymphocyte antigen 4 (CTLA-4), T cell immunoglobulin and mucin domain-containing protein (TIM3), programmed cell death protein (PD-1), or the ligand PD-L1, which can hijack policing immune checkpoints, allowing for immunoevasion.[63] HMOX1-induced gut dysbiosis plays a significant role in CRC progression and metastasis. However, it is currently less explored in the literature because this is a new development. Future studies are warranted, as gut microbiome modulation may significantly affect novel immunotherapeutics.

Heme oxygenase pathways as potential targets for inhibition of colorectal cancer

There is an increasing need for alternative anticancer treatments owing to the limitations of current therapeutic tools such as chemotherapy and radiotherapy. These therapies have been shown to increase oxidative stress in cancer cells by generating ROS to induce apoptosis.[58] However, this process activates antioxidant signaling systems, including upregulation of HMOX1, which promotes cancer cell survival and treatment resistance.[60] Therefore, exploiting HMOX pathways to reduce tumor burden is of significant interest for novel anticancer therapeutics [Figure 2].

Inhibition of HMOX1 has been shown to increase the susceptibility of cancer cells to apoptosis and improves sensitivity to cancer therapeutics.[58,60] The HMOX pathway offers multiple potential targets, including hijacking the HIF-1α/HMOX axis, Nrf2/HMOX axis, HMOX/CO axis, HMOX1-induced modulation of the tumor microenvironment, and HMOX expression in gut bacteria.

Pharmacological inhibition of HMOX1 via first-generation HMOX system inhibitors, metalloprotoporphyrins, or metallomesoporphyrins, interfere with the HIF-1α induced VEGF-mediated angiogenic pathway and can therefore limit tumor growth and spread.[66] Tumor progression is inhibited by the downregulation of associated metastatic proteins and pro-angiogenic mediator molecules such as VEGF and thymidine phosphorylase.[60] Apoptosis of tumor cells occurs due to the enhanced cytotoxicity of ROS accumulation in the absence of the antioxidative pleiotropic metabolites bilirubin and CO, thus leading to decreased tumor size.[34] Second-generation HMOX system inhibitors, such as imidazole-derived antifungal agents, are preferred over first-generation inhibitors because of their increased selectivity toward HMOX1 isoforms and fewer off-target effects.[60]

Overexpression of HMOX1 from excessive Nrf2 activity increases the expression of multidrug resistance-related proteins (MrPs), which contribute to chemoresistance.[58,67] In addition, approximately half of the distal CRCs involve mutations in the well-recognized tumor suppressor gene p53.[68] Reduced p53 activity has been correlated with the hyperactivation of Nrf2 signaling pathways.[69] Pharmacological targeting of the NrF2/Keap1 pathway through the positive modulation of Keap1 or inhibition of Nrf2 leads to growth inhibition and increased chemosensitivity. Furthermore, a recent study found that Nrf2 inhibition restores p53 activity and increases therapeutic resistance.[69] Inhibition of

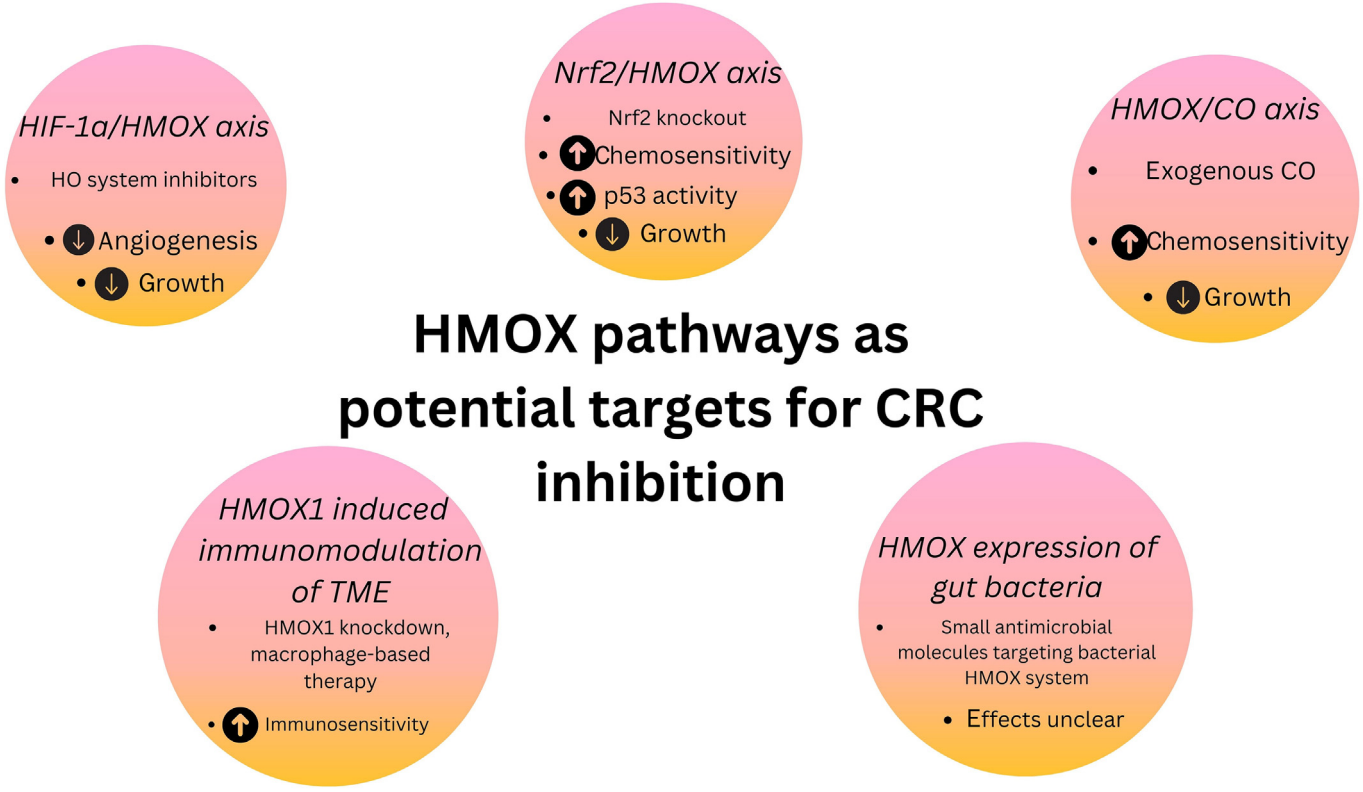

Figure 2. There are multiple potential targets in the HMOX pathway that could be exploited for CRC inhibition. This includes the HIF-1a/HMOX axis, Nrf2/HMOX axis, HMOX/CO axis, HMOX1-induced immunomodulation of TME, and HMOX expression of gut bacteria. The effects of targeting these HMOX pathways have been described in the illustration. CO: Carbon monoxide; HIF-1a: Hypoxia-inducible factor 1 alpha; HMOX: Heme oxygenase; Nrf2: Nuclear factor erythroid 2-related factor 2; TME: Tumor microenvironment.

Nrf2 may be achieved using pharmacological compounds that promote Nrf2 degradation or decrease Nrf2 nuclear translocation.[67] Small interfering RNA (siRNA) provides greater targeting precision than most nonselective HMOX1 inhibitors and has been found to induce cell death in the human colon cancer cell line HCT116 by specifically targeting Nrf2.[67] Nrf2 knockout in HCT116 colon cancer cells increased their susceptibility to the cytotoxic effects of cisplatin.[69] Interestingly, the antidiabetic biguanide metformin decreases Nrf2 expression, inhibits cell proliferation, and induces apoptotic cell death in HT29 CRC cells.[67]

Exogenous CO appears to disrupt the HMOX/CO equilibrium maintained in the tumor microenvironment, causing mitochondria-dependent generation of ROS.[42] The sensitivity of cancer cells to chemotherapy markedly increases after exogenous treatment with CO. This was demonstrated in PC3 prostate cancer cells, which were 1000-fold more sensitive to cytotoxic chemotherapy.[42]

Further studies have shown HMOX1 knockdown altered the impact of immune cells on the tumor microenvironment. The absence of HMOX1 causes a shift in T cell phenotypes, including reduced regulatory T cell involvement and increased Th1 cytokine production, thus reinstating a predominantly pro-inflammatory state.[48,60] This increases oxidative stress in malignant cells, leading to cell death.[60] Macrophage-based therapy involving the modification of patients' macrophages *in vitro* has been suggested as a potential immunomodulatory treatment.[43] Immunotherapeutics may play a significant role as novel anticancer treatments based on existing laboratory knowledge of HMOX1 induction effects in the tumor microenvironment. However, there is currently a deficit in translating this knowledge to clinical trials involving various immunomodulatory therapies for CRC.

Lastly, gut microbes, particularly Gram-negative bacteria, express an HMOX enzyme (HemO) that, despite its structural differences from human HMOX, can also metabolize heme. For example, *Neisseria meningitidis* and *Pseudomonas aeruginosa* HemO have less than 15% structural similarity with human HMOX. These differences in HMOX makeup suggest that small antimicrobial molecules that target bacterial HMOX systems may be useful in heme-induced dysbiosis.[60] However, the efficacy of this treatment remains unclear.

The lack of up-to-date studies in this field has narrowed the breadth of knowledge regarding the efficacy of HMOX1 inhibition as a novel anticancer treatment strategy for CRC. Most existing literature has focused on the role of HMOX1 inhibition in pancreatic and breast cancers. Although this could be perceived as helpful, it is apparent that the roles and behavior of HMOX1 are context-dependent on the tissue type and metabolic environment. Thus, the effects of HMOX1 inhibition on other cancer types may not translate to similar results in CRC. Therefore, further research on HMOX1 inhibition as a potential therapeutic tool for CRC needs to be conducted to determine whether exploiting these HMOX pathways produces meaningful data in that clinical context.

Controversy regarding heme oxygenase-1 as a potential tumor promoter

The clinical significance of HMOX1 in CRC pathogenesis and prognosis remains controversial. Several studies have indicated that high HMOX1 expression in CRC tumors leads to better survival outcomes.[44,70,71] The significance of these conflicting reports cannot be ignored, despite most existing literature demonstrating that HMOX1 functions in tumorigenesis, which is consistent with its role as a tumor promoter.

The multifaceted nature of HMOX1 appears to offer protagonistic and antagonistic roles depending on the environmental conditions. Prior to the tumorigenic threshold being reached, HMOX1 is upregulated to reduce oxidative stress in healthy colonic epithelial cells. Cytotoxic damage caused by high concentrations of intracellular heme increases the likelihood of carcinogenesis. Upregulation of HMOX1 is beneficial for increasing cancer cell survival via the mechanisms mentioned earlier in this manuscript and thus would lead to a poor prognosis. Additionally, it is important to consider that certain genetic variations in *HMOX1* are associated with an increased tendency for overexpression of HMOX1.[42] Polymorphisms involving an increased number of long (GT) repeats in the *HMOX1* promoter exhibit higher transcriptional activity and are associated with a higher incidence of gastrointestinal cancer.[47,48]

The explanation for the opposing outcomes associated with HMOX1 expression within the course of CRC development and progression remains unclear. Recently, Gamage et al. have found that low HMOX1 levels in a stage II colon adenocarcinoma cell line were associated with a significantly higher rate of lymphovascular invasion.[24] However, high HO-1 expression in advanced stages of CRC, such as stage III, is correlated with lymph node metastasis and reduced survival time.[42,48] Therefore, HMOX1 appears to work within a spectrum of activities along the disease continuum of CRC, possibly becoming increasingly pro-tumorigenic in the later stages of CRC. This may be due to the effects of prolonged chemotherapy or radiotherapy, which alters the cellular energetics and biological processes of the tumor microenvironment.

Conclusions

This study reviewed the pathological significance of HMOX1 in heme-induced CRC, with a detailed focus on the mechanisms underlying its protumoral capacity. A beneficial threshold exists in which HMOX1 provides cytoprotection to normal colonic epithelial cells under heme-induced oxidative stress. Once the carcinogenic process is established, the HMOX1 metabolite CO promotes tumor progression by maintaining abnormal cell proliferation pathways, inhibiting pro-apoptotic signals, and promoting metastasis through modulation of the immune surveillance system. In addition, dysregulation of the Nrf2/HMOX axis amplifies ROS detoxification in neoplastic tissues, which is an important survival factor for cancer cells. High heme levels suppress BACH1 activity leading to overactive Nrf2 transcriptional signaling, thus promoting the growth of established tumors. Furthermore, gut dysbiosis due to chronic inflammation causes the overgrowth of bacteria with analogous heme oxygenase activity. HMOX1 induction also modulates the tumor microenvironment. HMOX1 induction in myeloid-derived suppressor cells abrogates T cell cytotoxicity and promotes cell immortalization, development of a pre-metastatic niche, and increased resistance to immunotherapy. Targeting different aspects of the HMOX1 axis may lead to the development of novel anticancer treatments, particularly in cases of chemoresistance. Although HMOX1 appears to act as a tumor promoter in CRC, some studies have demonstrated that increased HMOX1 expression may lead to better survival outcomes. Therefore, HMOX1 may have a spectrum of activities in carcinogenesis, and further research is needed to clarify the role of HMOX1 expression at different stages of CRC.

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Author contributions

Rachitha Singhabahu conducted the literature search, write-up, editing, and created the figures. Sujani M. Kodagoda Gamage supervised and assisted in the write-up and creation of figures. Vinod Gopalan is the main supervisor of the work.

Ethics statement

Not applicable.

Data availability statement

The datasets used in the current study are available from the

corresponding author on reasonable request.

Conflict of interest

None.

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