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REVIEW ARTICLE

Importance of Heme Oxygenase-1 in Gastrointestinal Cancers: Functions, Inductions, Regulations, and Signaling

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Abstract

Introduction Colorectal cancer (CRC) is one of the important gastrointestinal tract tumors. Heme is mainly absorbed in the colon and induces nitrosamine formation, genotoxicity, and oxidative stress, and increases the risk of CRC.

Materials and Methods Information was collected from articles on Scopus, Google Scholar, and PubMed.

Results Heme can irritate intestinal epithelial cells and increases the proliferation of colonic mucosa. Heme can be considered as a carcinogenic agent for CRC induction. In typical situations, Heme Oxygenase-1 (HO-1) is expressed at low concentration in the gastrointestinal tract, but its expression is elevated during lesion and inflammation. Based on the multiple reports, the impact of HO-1 on tumor growth is related to the cancer cell type. Increased HO-1 levels were also indicated in different human and animal malignancies, possibly through its contribution to tumor cell growth, metastasis, expression of angiogenic factors, and resistance to chemotherapy. Recent studies noted that HO-1 can act as an immunomodulator that suppresses immune cell maturation, activation, and infiltration. It also inhibits apoptosis through CO production that leads to p53 suppression. The upregulation of HO-1 significantly increases the endurance of colon cancer cell lines. Therefore, it is supposed that HO-1 inhibitors could become a novel antitumor agent. *Lactobacillus rhamnosus* and its metabolites can activate Nrf2 and improves anti-oxidant levels along with upregulation of its objective genes like HO-1, and downregulation of NF-κB which reduce phosphorylated TNF-α, IL-1β, and PAI-1.

Conclusion The precise mechanism accountable for the anti-inflammatory features of HO-1 is not completely understood; nevertheless, the CO signaling function associated with the antioxidant property shown by bilirubin possibly will play an act in the improvement of inflammation.

Keywords Colorectal cancer · Heme oxygenase-1 · Microbiota · Inflammatory · Autophagy

Introduction

Colorectal cancer (CRC) is one of the important of cancer-associated death globally. Its prevalence rate varies among nations, with the most rate in developed countries and continuing growth in developing countries [1, 2]. Although the exact reasons for this increase are unclear, a positive family history, obesity, age, and environmental factors seem to have an association with the development of CRC [3]. Although approximately 75% of CRC cases are attributed to sporadic disease, mutations in specific genes including tumor suppressor genes (TSGs) like adenomatous polyposis coli (APC) and oncogenes, microsatellite instability (MIN), and chromosomal instability (CIN) can occur in CRC [4, 5]. According to the familial studies, only 12–35% of CRC cases are heritability. Based on the low level of heritability of CRC, it seems that environmental factors have the main role in CRC [6]. Diet pattern lifestyle and obesity have long been considered as the CRC risk factors [7, 8].

However, an altered metabolism as a hallmark of CRC can disturb symbiotic interaction between host and microbiota [9]. Gut microbiota is involved in several mechanisms such as induction of inflammation, genotoxin production, and immune regulation. Generally, imbalance in frequency and perturbation of the role of

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gut microbiota, have been related to CRC. *Enterococcus faecalis*, *Fusobacterium nucleatum*, *Helicobacter pylori*, and *Escherichia coli* are supposed to have a critical role in CRC induction via the production of reactive oxygen and nitrogen species (RONS), activation of inflammatory and oncogenic responses, and promotion of cell proliferation [10, 11]. Dietary Heme which is mainly absorbed in the colon induces nitrosamine formation, genotoxicity, oxidative stress, and enhances the risk of CRC [12]. Heme can irritate intestinal epithelial cells and increases the proliferation of colonic mucosa in rat models. Therefore, Heme can be considered as a carcinogenic agent for CRC induction [13].

HO-1 Activity and Isozymes

The cytoprotective enzyme family Heme oxygenase (HO) which is localized in the endoplasmic reticulum is induced by intestinal microbiota and various stress stimuli, including RONS, UV radiation, and hypoxia [14]. HO-1, HO-2, and HO-3 that are three isoforms of the HO with different tissue distribution, induction, and construction have been introduced. HO-1 and HO-2 genes are located in chromosomes 22 and 16, respectively [15]. HO has a significant role in the catabolism of Heme in the presence of three O_2 molecules and NADPH that cause the production of biliverdin (BV) that is later reduced to bilirubin, carbon monoxide (CO), and free iron (Fe^{2+}) in an oxidative cleavage manner [16].

HO-1 as a 32 kDa protein is greatly induced in the liver, bone marrow, and spleen in response to environmental stimuli [17]. However, in normal situations, HO-1 is expressed at low concentrations in the gastrointestinal tract, but its expression is elevated during lesion and inflammation [18]. Based on the multiple reports, the impact of HO-1 on tumor growth is related to the cancer cell type. It is supposed that the metabolic status of cancer cells influences how Heme degradation enzymes modulate tumor growth [19]. Enhanced HO-1 levels were also indicated in different human and animal malignancies, possibly through its contribution to tumor cell growth, metastasis, expression of angiogenic factors, and resistance to chemotherapy. Recent studies noted that HO-1 can act as an immunomodulator that suppresses immune cell maturation, activation, and infiltration. It also inhibits apoptosis through CO production that leads to p53 suppression [20]. The upregulation of HO-1 considerably increases the survival of colon cancer cell lines. Therefore, it is supposed that HO-1 inhibitors could become a novel antitumor agent [21].

HO-2 is a 36-kDa molecule that is constitutive expressed in the brain, endothelial, and reproductive cells. Cellular oxidative stress and NO sources can induce HO-2, although hypoxia could decrease its expression. Also, basal levels of HO-2 can protect against cellular stress and contribute to Heme homeostasis [22]. It is well known that HO-2 has a neuroprotective role in ischemia. However, data on the role of HO-2 in CRC are scarce and most of the actual knowledge on the HO system is related to HO-1 [23].

Important of HO-1 Inductions and Regulations

In physiological conditions, often tissues have a small amount of HO-1 expression, that is highly congested due to the removal of the older RBCs and therefore express high levels of HO-1. Also to being a substrate for HO-1, almost all stressful conditions can rapidly induce the expression of HO-1. A wide range of factors can stimulate HO-1 expression. In 1989, Tyrell and Keyes introduced HO-1 as an inducible protein [24]. Expression of this inducible isoform can be stimulated by a diversity of environmental factors, including nitric oxide (NO), cytokines, heavy metals, hormones, growth factors, thiol-reactive substances, oxidants, altered oxygen tension (hypoxia/hyperoxia), ischemia/reperfusion (I/R) injury, and ultraviolet (UV)-A radiation [17].

The nuclear factor erythroid 2 (NFE2)-related factor 2 (Nrf2) protein is the main region leucine zipper (bZip) transcription factor that mainly contributes to cellular defense against oxidative/electrophilic stress [25]. It acts as a transcriptional factor that controls a variety of metabolizing genes such as HO-1 at the transcriptional status. In physiological conditions, Kelch-like ECH-associated 1 (Keap1) protein binds to Cullin 3 (Cul3), a scaffold protein of Nrf2 ubiquitin ligase (E3), and restrains Nrf2 in the cytoplasmic Keap1/Nrf2/Cul3 complex [26]. However, in reaction to oxidative stress, Keap1 sulfhydryl groups are activated by chemical agents, cause to disruption of the Keap1/Nrf2/Cul3 complex and inhibition of Nrf2 ubiquitination. Therefore, Nrf2 is translocated into the nucleus, where it is attached to the antioxidant response elements (ARE) in the promoter region of genes related to antioxidant and detoxifying enzymes such as HO-1, Glucose-6-phosphate dehydrogenase (G6PD), and Gluthathione *S*-tranferases (GSTs) [27]. This pathway regulates the expression of growth factors, transcription factors, detoxifying molecules, and proteins that contribute to the protection of cells. Igarashi et al. proposed that the transactivation of Nrf2 can also be processed through direct binding of Heme to BTB (transcription repressor) and CNC homologue1 (Bach1) transcription factor that competes with Nrf2 binding region in the HO-1 promoter sites and leads to Nrf2/small Maf heterodimer formation [28]. In recent decades, it is considered that Nrf2 is upregulated in many kinds of cancers and plays a carcinogenic role in many organs. Dysregulation of Nrf2 can be mediated through several mechanisms, including genetic mutations,

epigenetic alterations, and stress conditions. The redox-dependent bach1/keap1/Nrf2 system is nearly related to the regulation of HO-1 expression. Nrf2 is the most important transcriptional regulator of HO-1 in response to inducible stimuli. Nrf2 is one of the families of Zip leucine transcription factors and is in control of the balance of cellular redox and antioxidant activity [29, 30]. In normal conditions, Nrf2 is inhibited by keap1 in the cytoplasm; these conditions contribute to the ubiquitination of Nrf2 by the Cullin-3-based E3 ubiquitination ligase complex. In this case, Nrf2 has a short half-life of about 20 min, providing low levels of Nrf2 to maintain cellular homeostasis. Therefore, protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK) phosphorylates not only EIF but also NRF2, both of which can ultimately play important roles in regulating the expression of HO-1 [31].

Nrf2/Stress Signaling

Nrf2 protects cancerous cells from oxidative stress leading to the formation and development of a tumor. Jung et al. described that activation of Nrf2 signaling through Keap1 silencing can attenuate oxidative damage [32]. Induction of oxidative stress via exposing human colorectal carcinoma cell line (HTC116 cells) to NO results in the transactivation of Nrf2. Mice null in Nrf2 fail to exacerbate anti-oxidative responses and are more sensitive to further colitis. During tumor cell growth, these cells encounter stress, owing to hypoxia conditions. At this time, Nrf2 is activated in response to oxidative substance accumulation. Based on Hu et al. results, the expression of Nrf2 is directly related to tumor size, lymph node metastasis, and progression to the advanced stage [33].

Intestine Microbiota and Nrf2

Although studies on the relation between Nrf2 expression and gut microbiome are scant, obviously Nrf2 is also vital for intestinal health and care. Interestingly, both symbiotic and pathogen bacteria trigger ROS generation in the gut epithelial cells, cause cell migration and proliferation through modification of the Nuclear Factor Kappa B (NF-κB) signaling pathway [34, 35]. In this manner, Nrf2 as a regulator controls the proliferation of gut stem cells [36]. Gut microbiota is also able to affect inflammation, through using the ubiquitin-proteasome pathway that controls subsequent signal transduction and immunity. Some bacteria such as *lactobacillus rhamnosus* GG (LGG) and its metabolites can activate Nrf2 and improves anti-oxidant levels along with upregulation of its objective genes like HO-1, and downregulation of NF-κB which reduce phosphorylated TNF-α, IL-1β, and PAI-1 [37, 38]. However, gut microbiota can be affected by HO-1 activity. Exposing germ-free-raised C57BL/6 mice to specific pathogen-free (SPF) promotes HO-1-related gene expression up to 6 times that needs to Nrf2 and IL-10. Therefore, it is considered that the Nrf2/Keap1 signaling pathway plays a vital role in host responses to microbiota stimuli and gut physiology. [39].

HO-1 as an Anti-inflammatory Factor

Recently, the anti-inflammatory effect of HO-1 has been revealed in CRC. Indeed, the anti-inflammatory impact of HO-1 through degradation of Heme molecules, the anti-oxidant function of bilirubin, and scavenging the free iron by ferritin has been reported by several studies [40]. As many studies have shown, HO-1 is considered to be a potent anti-inflammatory enzyme. HO-1's importance is shown by HO-1 knockdown investigations (using HO-1 −/− mice) that establish the advanced inflammatory disease and display significant increases in the rates of pro-inflammatory cytokines (IL-1β, IFN-γ, TNF, and IL-6). The precise mechanism accountable for the anti-inflammatory features of HO-1 is not completely understood; nonetheless, the carbon monoxide signaling function associated with the antioxidant property shown by bilirubin may have an important role in the improvement of inflammation [41].

Neurodystrophic and Neuroprotective Function of HO-1

As stated, the HO-1 expression is accountable for providing that neuroprotection under certain conditions. In a normal condition, brain HO-1 mRNA and protein expression are restricted to rare scattered neuroglia and neurons. HO-1 induction happens in response to diverse stress-causing factors. In Alzheimer's disease (AD), high expression of HO-1 protein happens in neurons, and astrocytes [42]. Also, in Parkinson's disease (PD), HO-1 is overexpressed in astrocytes [43].

Brain function largely depends on the oxygen source, because the brain consumes a large percentage of the total oxygen that enters the body. In normal conditions, 2–5% of total oxygen is used up by the cells and converted to ROS, so it can lead to some neurodegenerative diseases if there is a large and uncontrolled amount of ROS production in the CNS such as PD, AD, Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS). Nevertheless, there are currently indications that the expression of oxidase 1 aids prevents the pathogenesis of several neurological disorders. The induction of HO-1 was shown to play a neuroprotective function in disclosure to several noxious stimuli in animal models and tissue culture. Enhanced expression of HO-1 in neurons has been shown to protect them against oxidative stress begun by glutamate and H2O2. Moreover, HO-1, when overexpressed, defends neurons

from 1-methyl-4-phenylpyridinium (MPP)-mediated toxicity by cumulative the expression of neurotrophic glial cell-derived factor. The induction of HO-1 also influences other factors of inflammation. For example, in rat cortical astrocytes, the activity of HO-1 appears to be related to the amount of Prostaglandin E2 (PGE2) induction. In contrast, downregulation or knockout of HO-1 increases susceptibility to oxidative and other stress challenges [43].

Function of HO-1 in Cardiovascular Diseases

Ewing and his colleagues first demonstrated a principal role of HO-1 in preserving cardiac homeostasis by finding an improved HO-1 expression in the heart in reaction to hyperthermia. Furthermore, in studies of HO-1 knockout (HO-1 −/−) mice, hypoxia causes extreme right ventricular dilation and infarction relative to wild mice. Research has also used cardiac-specific transgenic mice to show that the size of myocardial infarction in subsequent ischemia/reperfusion injury has decreased because of increased expression of HO-1 [43].

HO-1 and Renal Function

The kidneys are exposed to a diversity of toxins, including endogenous and exogenous types. The released Heme is the most important molecule responsible for the stimulation of oxidative stress and ultimately induces HO-1. HO-1 induction has been revealed to happen in several kidney substructures like renal interstitium, tubules, mononuclear phagocytes, and glomeruli in reaction to damage. HO increases several-fold in angiotensin-II-treated mice, which induce renal injury, thereby protecting against hypertension. It has also been shown that gentamicin-mediated renal dysfunction can be efficiently remedied by persuading the expression of HO-1 [44].

Hepatoprotective Function of HO-1

Induction of HO-1 has been shown to protect the liver from various damages. A wide range of hepatotoxic conditions and chemicals can induce the expression of HO-1 and thus somehow protect the liver against injury [44]. Induction of HO-1 expression contributes to defense against liver injury induced by chemical composites like acetaminophen, carbon tetrachloride, and heavy metals, suggesting HO-1 induction as a significant cellular attempt for hepatoprotection [45].

HO and CRC: (Mechanisms of NRF2 Activation in CRC)

Activation of the Nrf2 response in normal or cancerous cells can lead to cell survival by their protection against oxidative stress, chemotherapeutic agents, and metabolic reprogramming (initiation of the anabolic pathway) [46]. Not surprisingly, in CRC tissues similar to any type of cancers, the raised levels of Nrf2 expression have been seen with high activity in the nucleus [47, 48]. As reported by Stachel et al., overexpression of Nrf2 due to an excessive level of ROS can lead to inflammation and initiation of tumorigenesis of colon tissues. Nrf2 overexpression reduces apoptosis and increases the proliferation of these cells [49]. Nrf2 expression is induced by external factors and drugs including oxidizing agents, allicin, Simvastatin, lipoic acid (LA), sulforaphane, and also via the repressor Keap1 in an indirect manner. However, mutations in *Nrf2* are also common evidence in cancerous cells [50].

There is some evidence that stimulation of Nrf2 leads to suppression of carcinogenesis in the early stages. Nrf2 can be induced by tumor suppressor genes such as BRCA1 and p21 protein [51]. Increased Nrf2 activity due to hypomethylation of the Nrf2 promoter has been observed in CRC, an event augmented chemoresistance. Also, it can promote autophagy and reduce inflammation and genotoxic harm in response to ROS elevation and p38 upregulation [52].

HO-1 and CRC

Takagi et al. showed that HO-1 mRNA is expressively increased in inflamed colonic mucosa [53]. Although Oláh reported a heterogeneous pattern of HO-1, expression in CRC may be reliant on the tumor subtype, stage, associated therapies, geographical origin of the patients, and other factors [54]. Based on the histological studies of colonic samples, HO-1 is primarily localized in inflamed mononuclear cells such as macrophages, and at low levels in epithelial cells [55, 56]. HO-1 acts as an anti-inflammatory function in the inflamed intestinal mucosa. It decreases the status of inflammatory cytokines like TNF-α and IFN-γ and also induces the expression of regulatory cytokines like IL-4 and IL-10 [41]. It is also revealed that the induction of HO-1 inhibits the expression of adhesion molecules, including vascular cell adhesion protein (VCAM), intercellular adhesion molecule (ICAM), and E-selectin, therefore restrictive the relocation of inflammatory cells [57]. Engagement of the CX3CR1 receptor on intestinal macrophages leads to IL-10 production through HO-1 upregulation and resolves inflammation in the intestine [58]. However, the function of HO-1 in gut malignancies remains to be illuminated. A growing body of evidence reports that HO-1 expression increases in CRC-tumor tissues. According to Seo et al., the upregulated HO-1 expression in CRC cells distinctly reduced the migration of PBMLs and their cytotoxicity power in contrast to CRC cells [59]. Also, HO-1 has a pro-angiogenic potent that support tumor progression [60, 61]. Whereas Becker et al. indicated that the upregulated range of HO-1 in CRC and adenoma reduces lymphatic invasion and metastases and is associated

with better outcomes [62]. However, most functions of HO-1 are present well-known to be arbitrated by CO. Similarly, the circulation levels of CO increase in CRC. Administration of low levels of exogenous CO reduced intestinal inflammation through downregulation of NO generation, tumor necrosis factor alpha (TNF-α), and IL-6 production in LPS-stimulated macrophages and $CD4^+$ T cells [63], and reduction of tissue-associated myeloperoxidase (MPO) activity that suppresses neutrophil infiltration into colonic mucosa [41]. It has been shown that CO can affect cancer metastasis, particularly to the liver [64]. Also, circulation levels of CO increased in C26 solid tumor-bearing mice in parallel through HO-1 activity. Contradictory, a current study presented that exogenous CO inhibits the development of prostate cancer through a different approach than HO-1 to induce the metabolic exhaustion of tumor cells. Generally, it seems that the roles of CO in a tumor are a double-edged sword [65].

HO-1, Autophagy, and CRC

Autophagy as a genetically regulated homeostatic program has a vital role in cell security against stress conditions like oxidative stress, ER stress, hypoxia, and starvation. In this process, denaturated-cell organelles are isolated in double-membraned sections and then fused to lysosomes which provide a source of energy for cell maintenance. Although an appropriate autophagy response is essential for genome and mitochondria stability owing to its ability to remove mutagens and redox-active aggregates, its activation in hypoxia areas of tumor improves cell survival. The numerous tumor suppressor and activator genes are involved in the autophagy process, with microtubule-associated protein 1 light chain 3 (LC3), autophagy-related genes (Atgs), Bax-interacting factor 1 (Bif-1), and beclin-1 genes [66].

Indeed, there is paradoxical evidence about the impact of HO-1 on autophagy in several cancers. Banerjee et al. reported that high expression of HO-1 induced survival of tumor cells through induction of anti-apoptotic B-cell lymphoma-extra-large (Bcl-xL) protein and prevention of autophagy in renal cancer [67], whereas the upregulation of HO-1 in MDA-MB-231 and MDA-MB-453 was associated through detectable increases in cytoprotective autophagy via the Scr-STAT3 signaling pathway [68]. The knockdown of HO-1 using two strands of siRNAs significantly increased apoptosis in MKN-28 and SGC7901 cells (gastric cancer lines) [69]. As reported by Cernigliaro et al., activation of the Nrf-2/HO-1 antioxidant response in CRC-tumor cells can help tumor survival by producing an ideal environment for cell development [70]. Treatment of CRC cell lines (HT-29, Caco-2, and HT116) with ethanol elevated ROS, iNOS, and COX2 levels and induced oxidative and ER stress. ER stress can initiate autophagy via phosphorylation of eIF2α to induce the beginning of LC3 [71], in addition to growing the level of beclin and atg7 in CRC cells. In this manner, Nrf2 is translocated to the nucleus, possibly through phosphorylation of Nrf2 by PERK- a kinase activated following the gathering of unfolded proteins during ER stress condition that resulting in the detachment of Nrf2/Keap1 complexes and Nrf2 nuclear translocation [72]. Following Nrf2 activation, several genes including catalase and HO-1 are overexpressed and promote uncontrolled cell proliferation, angiogenesis, and poor prognosis, as can be seen in primary CRC and metastatic tissues. Nevertheless, little information is available about the role of HO-1/autophagy in CRC. [52, 70].

Ubiquitination/Proteasomal Degradation and HO-1 in Cancer

In current years, researches about yeast and mammalian cells have discovered that many ER-associated aberrant and typical proteins are degraded by the ubiquitin-proteasome system via ER-associated degradation (ERAD) processes [73]. It has been revealed that the ubiquitin-mediated proteolytic way is accountable for the non-lysosomal degradation of the common intracellular proteins [74].

ERAD protein molecules primarily undertake ubiquitination in the ER membrane via the synchronized act of three enzymes, ubiquitin-activating enzyme E1, ubiquitin-conjugating E2, and ubiquitin ligase E3. E3 ligases provide the substrate specificity essential for protein ubiquitination. So far, three different classes of E3 ligases have been recognized. The HECT ligases, which contain a domain homologous to E6AP C-terminus, form thiol-ester interact with ubiquitin and then transfer it to the protein substrate. Despite this, the U-box-containing E3 ligases and zinc-binding RING-E3 ligases can mediate the direct transfer of ubiquitin from E2 to substrates [75, 76].

HO-1 is the substrates for the ubiquitin-proteasome system [77]. The mechanism underlying the regulation of steady-state HO-1 turnover, the current study discovers the role of the ubiquitin-proteasome system in the degradation of HO-1 and the potential involvement of ERAD in this procedure [78]. In the current study, we established that proteasome inhibition meaningfully attenuated the degradation of both endogenous and exogenous HO-1 and improved the accumulation of exogenous HO-1, signifying the participation of the ubiquitin-proteasome system in HO-1 turnover [78]. This opportunity was further maintained by a series of test presentation HO-1 ubiquitination [79].

Yerlikaya observed the expression of HO-1 in p53-null breast cancer and p53-wt melanoma cell lines in response to the proteasome inhibitors MG132 and bortezomib. HO-1 is strongly induced in these cell lines after a limited incubation with MG132 or bortezomib. Nevertheless, the expression

of HO-1 was not induced in p53- deficient cells at the same time. This recommended that HO-1 expression is under the control of p53 [80].

Also, low doses of MG132 induced HO-1 expression in human vascular cells, and the antioxidant transcription factor Nrf2 was crucial for MG132-mediated induction of HO-1 [81].

At the Yerlikaya studies, they observed that treated cells for long periods with bortezomib, HO-1 induction of HO-1, were detected in both p53-wt and p53-deficient cells. It is supposed that induction of HO-1 in p53-null cells observed after 24 treatments with bortezomib may be managed by Nfr2, having a comparatively extensive half-life than p53. The notable induction of antiapoptotic and cytoprotective HO-1 seemingly decreases the efficiency of proteasome inhibitor bortezomib, which is appropriate for multiple myeloma, recurrent multiple myeloma, and mantle cell lymphoma. So, it is believed that further description of the mechanisms causing the up-regulation of HO-1 under the conditions of proteasomal inhibition may lead to the design of more effective cancer treatment approaches [80].

Conclusion

HO-1 is a critical and rate-limiting enzyme in the catabolism of the Heme molecules. In the enzymatic degradation of Heme by HO-1, the alpha-methane bridge is broken in the ring structure and the molecules of bilirubin, CO, and ferrous iron as reaction products are created. In general, all products resulting from HO-1 activity take part in physiological procedures like oxidative stress, inflammation, and apoptosis. Biliverdin is converted to bilirubin through the act of biliverdin reductase. The biliverdin and bilirubin bile pigments will scavenge ROS and NRS through a recycling mechanism. Bilirubin has also been suggested to suppress inflammatory responses and reduce cellular toxicity. CO, as another by-product of co-oxygenating enzymatic activity, can induce anti-apoptotic, anti-inflammatory, and anti-proliferative properties by modulating MAPK, p38 pathways. CO stabilizes HIF1, which plays a protective effect in macrophages and can prevent cytochromes of the respiratory chain and NADPH oxidase (NOX), thus attributed to the reduction of ROS. Ferrous iron is one of the products of the HO-1 system which can be rapidly removed by ferritin to prevent prooxidant capacity. In general, the Heme molecule is highly hydrophobic and can simply bind to lipids, leading to the peroxidation of membrane lipids and this can damage the membrane of some cellular organs, including the ER, nucleus, and cell membrane. The HO-1 system can retain the Heme protein in a healthy state and protect the cells from free Heme injury inside the cell. Therefore, the cytoprotective function of the HO-1 system in biological processes is very essential.

Recent studies noted that HO-1 can act as an immunomodulator that suppresses immune cell maturation, activation, and infiltration. It also inhibits apoptosis through CO production that leads to p53 suppression. The upregulation of HO-1 significantly increases the survival of colon cancer cell lines. Therefore, it is supposed that HO-1 inhibitors could become a novel antitumor agent. *Lactobacillus rhamnosus* GG and its metabolites can activate Nrf2 and improves anti-oxidant levels along with upregulation of its goal genes like HO-1, and downregulation of NF-κB which reduce phosphorylated TNF-α, IL-1β, and PAI-1. The precise mechanism accountable for the anti-inflammatory properties of HO-1 is not completely understood; however, the CO signaling function associated with the antioxidant property shown by bilirubin possibly will play a role in the improvement of inflammation.

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