บทบาทของธาตุเหล็กฮีมต่อการเกิดมะเร็งลำไส้ใหญ่ที่มีการกลายพันธุ์ของยีน *APC* และกลยุทธ์ทางเลือกในการป้องกันมะเร็งลำไส้ใหญ่

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บทคัดย่อ

ในปี 2020 มะเร็งลำไส้ใหญ่และทวารหนักเป็นมะเร็งที่พบมากเป็นอันดับสามและเป็นสาเหตุอันดับสองของการเสียชีวิตจากโรคมะเร็งทั่วโลก การกลายพันธุ์ของยีน adenomatous polyposis coli (*APC*) เป็นหนึ่งในปัจจัยของการเกิดมะเร็งผ่านการรบกวนสัญญาณ Wnt โดยธาตุเหล็กฮีม (heme iron) ซึ่งเป็นส่วนประกอบหลักของเนื้อแดงสามารถกระตุ้นภาวะเครียดทางออกซิเดชันและการอักเสบเรื้อรัง ปัจจัยทั้งสองเหนี่ยวนำให้เกิดการส่งสัญญาณ Wnt และส่งผลให้เซลล์มีการเจริญเติบโตผิดปกติ อย่างไรก็ตาม ความเป็นพิษต่อเซลล์ที่เกิดจากธาตุเหล็กฮีมไม่ส่งผลต่อเซลล์ที่มีการกลายพันธุ์ของยีน *APC* ทำให้ความเสี่ยงของการเกิดมะเร็งของเซลล์กลายพันธุ์เพิ่มขึ้น กลไกที่เกิดขึ้นได้แก่ การเปลี่ยนแปลงทางชีวภาพของสารจากปฏิกิริยาออกซิเดชันของลิพิดที่มีประสิทธิภาพมากขึ้น การเพิ่มการสังเคราะห์เอนไซม์ต้านอนุมูลอิสระผ่าน nuclear factor erythroid 2-related factor 2 (Nrf2) และการลดการแสดงออกของ heme oxygenase-1 กระตุ้นการอักเสบ นอกจากนี้ ธาตุเหล็กฮีมยังรบกวนสมดุลของจุลินทรีย์ในลำไส้ ทำให้เกิดการอักเสบเรื้อรังร่วมกับการเติบโตของเซลล์ที่เพิ่มมากขึ้น อนึ่ง เซลล์ที่มีการกลายพันธุ์ของยีน *APC* สามารถลดการตายของเซลล์โดยผ่านการรบกวนสัญญาณการอักเสบนิวเคลียร์แฟคเตอร์-แคปปาบี (nuclear factor kappa B) ด้วยเหตุนี้ เพื่อลดความเสี่ยงของการเกิดมะเร็งลำไส้ใหญ่โดยเฉพาะผู้ที่มีการกลายพันธุ์ของยีน *APC* ควรจำกัดการบริโภคอาหารที่อุดมด้วยธาตุเหล็กฮีม และอาจพิจารณาเพิ่มการบริโภคผลิตภัณฑ์อาหารเสริมที่ให้ประโยชน์ในการต้านอนุมูลอิสระ ต้านการอักเสบ และการปรับสมดุลของจุลินทรีย์ในลำไส้

คำสำคัญ: ธาตุเหล็กฮีม มะเร็งลำไส้ใหญ่และทวารหนัก ยีน *APC* จุลินทรีย์ในลำไส้ วิถีสัญญาณ Wnt

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Role of Heme Iron in the Progression of Colon Cancer with *APC* Gene Mutated and Alternative Strategy for Colon Cancer Prevention

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Abstract

In 2020, colorectal cancer was the third-most prevalent cancer and the second-leading cause of cancer-related deaths worldwide. The adenomatous polyposis coli (*APC*) gene mutation promotes cancer progression by disrupting Wnt signaling. Heme iron, the fundamental component of red meat, can trigger oxidative stress and chronic inflammation, provoking Wnt signaling and resulting in excessive cell growth. Nevertheless, the cytotoxic effect of heme iron is rendered ineffective toward *APC*-mutated cells, increasing the risk of cell hyperproliferation. *APC*-mutated cells possess more efficient biotransformation of lipid peroxidation products and highly express nuclear factor erythroid 2-related factor 2 (Nrf2), resulting in augmented antioxidant enzymes. While nuclear Nrf2 levels are raised in *APC*-mutated cells, the expression of heme oxygenase-1 is reduced compared to wild-type cells, thereby causing an increase in the proinflammatory process. In addition, *APC*-mutated cells escape apoptosis by impairing the nuclear factor kappa B inflammatory pathway. Heme iron further enhances chronic gut inflammation by causing an imbalance in the intestinal microbiota, thereby increasing cell growth. To minimize the risk of colon cancer, especially for those with an *APC* gene mutation, limiting the consumption of foods rich in heme iron such as red meat is advisable. Additionally, considering dietary supplements that provide antioxidant, anti-inflammatory, and gut microbiota modulation benefits could also be beneficial.

Keywords: Heme iron, Colorectal cancer, *APC* gene, Gut microbiota, Wnt signaling pathway

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Introduction

Colorectal cancer (CRC) is the third-highest cancer incidence and the second-leading cause of cancer-related deaths among the global population in 2020[1]. Genetic factor is one of the main contributors to cancer progression. In 50-70% of colon cancer cases, the traditional pathway of colon cancer progression initiates from Adenomatous polyposis coli (*APC*) gene mutation followed by the role of Kirsten rat sarcoma viral oncogene homolog (*KRAS*) proto-oncogene. The mutation of *APC* gene has been reported in Aberrant crypt foci (ACF), a cluster of abnormal crypts that line the colonic mucosal surface and are viewed as the earliest morphological change of precancerous lesion in colon cancer[2-4], and is strongly associated with familial adenomatous polyposis (FAP), a widely known inherited disorder that can lead to a large number of colorectal polyps and an increased likelihood of developing cancer[5]. APC protein has been well recognized as a regulator of the Wnt signaling pathway by promoting β-catenin turnover[6]. The accumulation of β-catenin overactivates the transcription of Wnt target genes involving cell proliferation and cell cycle regulation, thereby enhancing tumor progression[7]. Since oxidative stress and chronic inflammation can activate the Wnt signaling pathway[8,9], prooxidant and proinflammatory agents may amplify colon cancer risk, particularly in those with *APC* gene mutation.

Red meat consumption increases the risk of colon cancer by 17% in the recent systematic review and meta-analysis study[10] and is positively associated with *APC* gene mutation[11-13] and ACF formation[14,15]. Heme iron, a porphyrin ring binding with iron predominantly derived from hemoglobin and myoglobin of red meat, is not only strongly related to an increased risk of CRC with *APC* gene mutation among the Netherlands's population[11], but those from five cohort studies worldwide with a higher intake of heme iron also have a significantly greater risk of colon cancer compared to the lower intake group[16]. Heme iron can act as a prooxidant, promoting DNA damage and increasing cell proliferation[17]. Moreover, it has been suggested that the disruption of the gut microbiota (GM), also known as gut dysbiosis, caused by the consumption of red meat and heme iron is linked to the development of CRC as a result of prolonged gut inflammation, impaired intestinal barrier, and ultimately increased cell proliferation[18-22]. Nevertheless, the dietary suggestion for cancer prevention only recommends to lower red meat intake due to its high-biological value proteins and numerous essential nutrients, replacing red

meat with a nutrient-rich alternative food source remains a challenge[23].

Given that reducing exposure to a modifiable risk factor, such as diet, has a greater impact on preventing colon cancer than non-modifiable risk factors[24], it is essential to understand the oncogenic effects of heme iron to reduce the risk of cancer development. Despite the evidence that heme iron may act as an alkylating agent on the *APC* gene, leading to gene mutation and sporadic cases[11], less attention has been dedicated to how *APC*-mutated cells respond to heme iron. This insight can be employed to develop an alternative strategy to prevent colon cancer in individuals with *APC* gene mutation or those with first-degree relatives diagnosed with FAP. This review, thereby, aims to update the current evidence of heme iron-related colon tumorigenesis, focusing on the potential involvement of the *APC* gene.

APC gene and colon cancer

1. Overview

The major three molecular genetic pathways of colon cancer include microsatellite instability, CpG island methylator phenotype, and chromosomal instability (CIN), which is responsible for nearly 70% of all colon cancer cases[4,25]. Karyotypic abnormalities are a hallmark of CIN, consisting of aneuploid karyotype, loss of heterozygosity, and chromosomal rearrangements[4]. The mutation of the *APC* gene has been reported as the earliest genetic event of colon carcinogenesis and has been observed in up to 70% of CIN-related colon cancer cases[26] and 80% among sporadic cases[7].

The *APC* gene, located on chromosome 5q21–22, encodes a large multi-domain protein, containing multiple binding sites for several proteins, including IQ-motif-containing GTPase activation protein 1, PP2A, Asef, KAP3, β-catenin, Axin, microtubule, and EB1. The *APC* gene is consequently essential for cell proliferation, cell adhesion, cell differentiation, and chromosome segregation[7]. Unfortunately, more than 98% of *APC* gene mutations are frameshift or nonsense mutations, creating a premature stop codon. Truncated or shortening protein is synthesized, accordingly[5]. Apart from the binding sites of β-catenin and Axin, the missing of the microtubule and microtubule-associated protein EB1 interacting sites is typically found in the C-terminally truncated APC protein. Owing to the significant role of microtubules in chromosome segregation, the mutated *APC* gene increases CIN, thus posing a greater risk of colorectal tumorigenesis[7].

2. The role of Wnt signaling pathway and APC gene mutation on colon tumorigenesis

Under normal circumstances, the intestinal epithelium is continuously replenished by the division of intestinal stem cells (ISCs) residing in intestinal crypts. The Wnt signaling pathway is crucial for intestinal cell proliferation and is well-regulated by various mechanisms. A destruction complex, consisting of scaffolding protein Axin, APC, protein phosphatase 2A, casein kinase I (CK1), and glycogen synthase kinase 3β (GSK3β) is the primary intracellular regulator of canonical Wnt signaling pathway. In the presence of Wnt ligands or Wnt-on state, the transmembrane complex, including Frizzled and low-density lipoprotein receptor-related protein 5/6, is enabled and the protein Disheveled is activated afterward[27,28]. Consequently, the Axin is recruited and interacts with the protein Disheveled, downregulating the destruction complex and promoting β-catenin accumulation in the cytoplasm[29]. The stabilized β-catenin later translocates into the nucleus and binds with the transcription factor Lymphoid Enhancer Factor/T-Cell Factor, inducing the transcription of Wnt/β-catenin target genes and, ultimately, promoting cell proliferation. When Wnt signaling is inactive, cytosolic β-catenin is first phosphorylated by CK1 on Serine (Ser) 45 and then further phosphorylated by GSK3β on Ser33, Ser37, and threonine 41, causing β-catenin to be ubiquitinated and degraded. Hence, the Wnt/β-catenin signaling pathway is well-regulated by a destruction complex[27,28]. Since the APC protein contains the multiple binding sites of β-catenin and Axin, it is unsurprising that the truncated APC protein impairs the function of the β-catenin destruction complex[6], leading to the β-catenin accumulation and the overexpression of Wnt/β-catenin target gene transcription[26]. Proto-oncogene cyclin D1 and c-myc are one of the target genes and critical for regulating cell proliferation[28]. The dysregulation of these proto-oncogenes has been hypothesized to be a contributory mechanism of colorectal carcinogenesis resulting from the Wnt/β-catenin signaling pathway[30,31]. Intriguingly, the accumulation of β-catenin has been reported in dysplastic ACF[2,3], a noteworthy biomarker of early colon carcinogenesis[32], pointing to the role of imbalanced Wnt/β-catenin signaling pathway in colon tumorigenesis.

The majority of colon cancer develops from *APC* gene mutation followed by *KRAS* proto-oncogene. After tubular adenoma is formed, the mutation of tumor protein 53 subsequently induces adenocarcinoma[4]. The mutation of the *APC* gene

has been reported in ACF of both human and murine[33-35] and is more highly presented in adenomas, a precancerous lesion of colon cancer, compared to the *KRAS* gene mutation[35,36]. Likewise, both ACF formation and cancer development are found in *APC* mutant mice with multiple intestinal neoplasms (*Apc*$^{Min/+}$ mice) along with β-catenin accumulation, while only ACF is observed in mice with *KRAS* gene mutation[37]. Ultimately, acute *APC* gene silencing yields an elevation of non-phosphorylated β-catenin, consequently leading to the upregulation of c-myc. The development of colonic polyps in mice with *APC* gene suppression has been further verified. Notably, these consequences are reversed upon *APC* gene reactivation[38]. These findings strongly support the importance of the *APC* gene and Wnt/β-catenin pathway homeostasis on colon tumorigenesis. The chemopreventive effects of phytochemicals focusing on the Wnt pathway have been extensively investigated in the past decade[39]. Nonetheless, greater attention should be dedicated to the negative effects of dietary factors on the Wnt pathway to minimize the possibility of colon tumorigenesis, particularly in those with *APC* mutation[40].

3. Heme iron and colon cancer

3.1 Overview

Red meat, unprocessed mammalian muscle meat, has been categorized as a probably carcinogenic agent to humans since 2015 by the International Agency for Research on Cancer. Several genotoxic compounds generated during the cooking process of red meat have been suggested as possible contributors to colon cancer, such as N-nitroso compounds, heterocyclic aromatic amines, and polycyclic aromatic hydrocarbons[41]. In addition to these compounds, animal fat and heme iron, which are the fundamental components of red meat, have been found to play a role in colon tumorigenesis. However, several recent studies report no association between dietary fat and CRC risk[42,42]. On the other hand, the individuals with a higher intake of heme iron have an elevated risk of colon cancer 1.18 times compared to the lower intake group[16]. Several hypotheses have been proposed to explain the mechanisms of heme iron-driven colon carcinogenesis, including DNA damage from oxidative stress and lipid peroxidation, chronic inflammation, and hyperproliferation of colon epithelial cells.

Dietary iron is generally presented in 2 forms, including heme iron and non-heme iron. Compared to non-heme iron, heme iron shows a higher bioavailability

and is mainly unaffected by other nutrients on absorption. After exposure to a low-pH environment and proteolytic digestive enzymes, heme is detached from hemoglobin and myoglobin. Since the majority of heme is moderately absorbed in the duodenum, up to 90% of heme is delivered to the large intestine. Although there is still no confirmation on the mechanisms of heme absorption, heme is likely to enter enterocyte via receptor-mediated endocytosis, located on the top of microvilli[44,45].

3.2 The underlying mechanisms of heme iron-related colon tumorigenesis and its correlation with APC gene mutation in carcinogenesis

- ***Oxidative stress and lipid peroxidation***

After heme uptake, cytosolic heme is cleaved by heme oxygenase-1 (HO-1) with electron transferring from NADPH-cytochrome P450 reductase. Carbon monoxide, ferrous iron (Fe^{2+}), and biliverdin are generated, accordingly[44,45]. Although carbon monoxide and biliverdin have anti-inflammatory and antioxidant properties, respectively, hydrogen peroxide, one of the well-known reactive oxygen species (ROS), is formed during heme degradation by HO-1[46]. In the presence of ferrous iron, hydrogen peroxide is catalyzed and yields hydroxyl radical, the most highly reactive free radical. Hydroxyl radicals can rapidly interfere with the surrounding environment and target the DNA strand, causing DNA strand break[47]. In human intestinal epithelial Caco-2 cells, the presence of heme causes DNA damage, and the degree of DNA strand break is decreased in a dose-dependent manner when treated with Zn(II) protoporphyrin IX (ZnPP), a competitive inhibitor of HO-1, and catalase, an antioxidative enzyme. Apart from the heme-related DNA damage, Caco-2 cell proliferation is remarkably increased by 45% with the heme concentration of 1 mM. As expected, the percentage of cell proliferation is significantly reduced by ZnPP and catalase[17]. These findings suggest the potential mechanisms of heme-induced colon carcinogenesis through oxidative stress promoting DNA damage and increasing cancer cell proliferation.

Hydroxyl radicals not only damage the DNA strand, but also favorably attack polyunsaturated fatty acids (PUFAs) or molecules containing PUFAs, e.g., phospholipids. Once lipid peroxidation is initiated, lipid radicals swiftly react with oxygen and generate lipid peroxyl radicals. Then, the lipid peroxyl radicals remove hydrogen from another non-radical lipid molecule for their stability, and a new lipid radical is created afterward. After receiving hydrogen, lipid hydroperoxide is developed

and known as a primary lipid peroxidation product. 4-hydroxynonenal (4-HNE), the most active lipid peroxides, and malondialdehyde (MDA) are the most widely studied aldehyde-containing secondary products of lipid peroxidation. 4-HNE acts like a second messenger for signal transduction, and at the same time, it can abruptly react with thiols and amino groups, resulting in protein adduct. The presence of MDA activity results in protein adducts, similar to 4-HNE[47,48].

In murine studies, the correlation between heme concentration in fecal water and heme component in food sources is demonstrated after red meat treatment. The level of thiobarbituric acid reactive substances (TBARS) assay, the method measuring MDA level, is also correlated with heme concentration in fecal water[14,49]. Interestingly, while the apoptosis of a normal intestinal cell line is induced by fecal water rich in lipid peroxidation-derived aldehydes, the *APC*-mutated colon epithelial cells are resistant to the cytotoxicity effect of lipid aldehydes by weakening caspase-3 activity and, thus, suppressing cell apoptosis[50]. On top of that, the *APC*-mutated cells also demonstrate more efficiency in the biotransformation of 4-HNE, leading to less cytotoxicity in mutant cells[51]. Therefore, these exceptional capabilities of the *APC*-mutated cells indicate a strong potential to be a leading cause of heme iron-related colon tumorigenesis in those with *APC* mutation.

- ***Dysregulations of antioxidant defense mechanism and chronic inflammation***

Under normal circumstances, a low level of 4-HNE induces nuclear factor erythroid 2-related factor 2 (Nrf2) gene expression, a transcription factor related to antioxidant enzymes. Conversely, an excessive 4-HNE reverses this protective effect followed by apoptosis, necrosis, and eventually cell death[47,48]. To against oxidative damage, the nuclear translocation of Nrf2 in colon epithelial cells is elevated after exposure to the fecal water of both dietary hemoglobin-fed and beef-fed rats and 4-HNE. However, a higher level of nuclear Nrf2 is observed in *APC*-mutated colon epithelial cells, implying that the increased antioxidant defense mechanism in preneoplastic colon cells may play a role in colon carcinogenesis (Figure 1). Intriguingly, the activation of Nrf2 is diminished with the absence of carbonyl compounds, including aldehydes, in fecal water[52]. Collectively, these findings high-light the importance of maintaining redox balance in both normal colon epithelial cells and *APC*-mutated cells to prevent heme iron-related colon tumorigenesis.

On top of modulating oxidative stress, Nrf2 is also critical for regulating chronic inflammation, which can contribute

to CRC, by increasing the expression of HO-1. In spite of an increased nuclear Nrf2 in colon epithelial cells with *APC* mutations, HO-1 expression is found to be lower in *APC*-mutated cells than in wild-type colon epithelial cells when exposed to heme-rich fecal water[52]. Taken together, the greater antioxidant capacity and the lessened anti-inflammation in *APC*-mutated cells may be a contributing factor to CRC carcinogenesis.

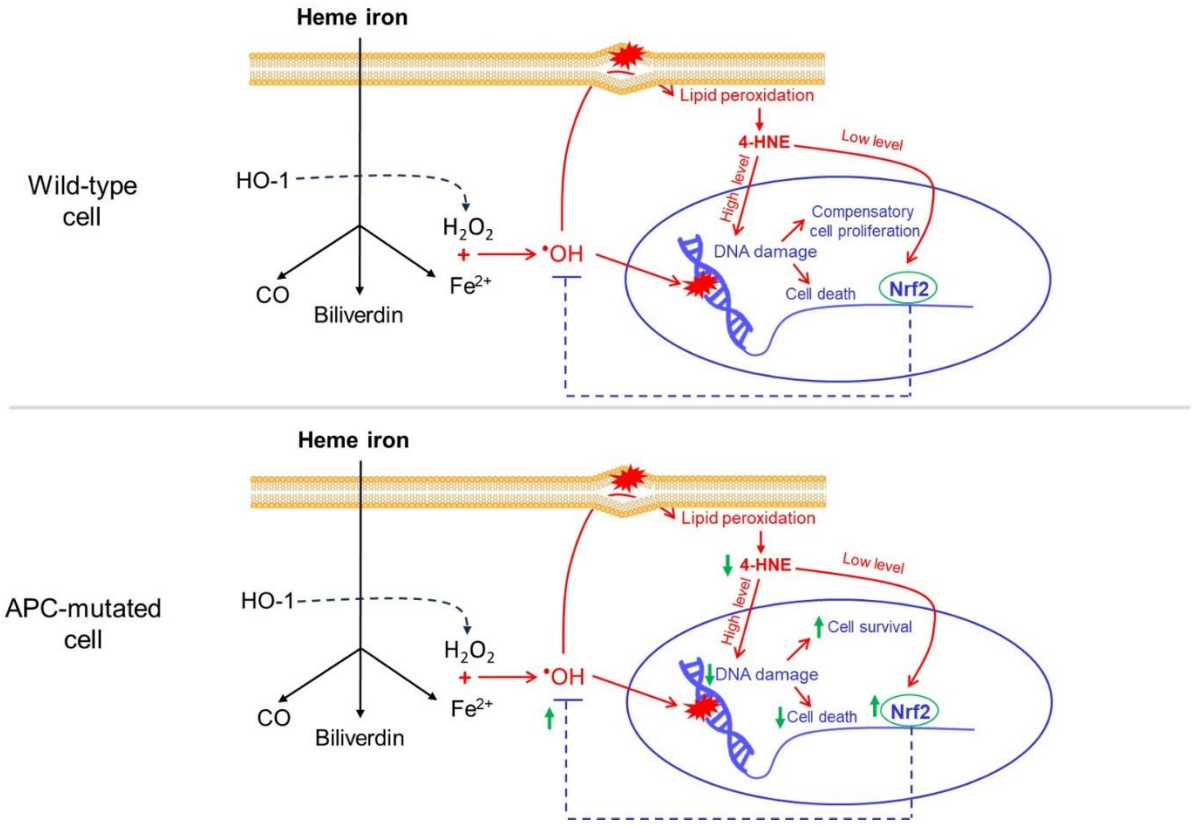

Figure 1. The molecular mechanisms of heme iron-driven colon tumorigenesis via oxidative stress and lipid peroxidation in wild-type and *APC*-mutated cells

- ***Cell hyperproliferation***

The clinical manifestations of colon carcinogenesis, i.e., ACF formation, are reported in rats fed with beef and blood sausage. Both beef diet and blood sausage, which contain a higher heme content than beef, promote ACF formation in a heme-dependent manner in rats treated with carcinogen azoxymethane (AOM)[14]. The number of ACF is also significantly increased with a beef diet in rats treated with carcinogen 1,2-dimethylhydrazine (DMH), confirming the clinical role of heme-induced colon carcinogenesis[49]. According to the aforementioned mechanism, the Wnt signaling pathway is of critical importance in intestinal epithelial

cell (IEC) proliferation. Interestingly, Wnt inhibitory factor 1 (Wif1), a negative regulator of Wnt signaling, is 5 times downregulated in mice treated with 0.5 μmol/g of heme. Consequently, crypt cell proliferation is promoted in heme-fed mice[53].

The time course of heme-induced colon epithelial hyperproliferation is investigated in C57Bl6/J mice receiving 0.2 μmol heme/g with low calcium diet. The markers of oxidative stress and lipid peroxidation is first elevated after heme exposure. The expressions of protein-related cytotoxic stress and cell death are upregulated after 4 days, in parallel with downregulated Wif1 resulting in compensatory hyperproliferation of colon epithelial cells[54]. The potential effects of heme on promoting colon carcinogenesis are summarized in Table 1.

Collectively, heme iron can induce cell hyperproliferation by inhibiting Wif1, eventually activating the Wnt/β-catenin signaling pathway. Those possessing the *APC* gene mutation are more likely to be susceptible to colon cancer when consuming heme. Despite this, a rapid increase in cell growth occurs after being exposed to oxidative stress and lipid peroxidation. Nutrients having antioxidant activity may provide a defensive role against heme-induced colon carcinogenesis.

- ***Gut dysbiosis and chronic gut inflammation***

In terms of diet, GM has been well acknowledged for its interplay with the host diet on chronic disease progression[55]. Lipopolysaccharide (LPS), the principal membrane component of gram-negative bacteria (GNB) and generally referred to as an endotoxin, plays a critical role in GM-related chronic inflammation. Following a 14-day heme diet, the GNB shows a substantial rise in relative abundance to 66.6±3.2% out of all the GM identified, and the GNB to gram-positive bacteria (GPB) ratio is notably higher than that of the control group. Although the heme-rich fecal water has no effect on GNB, the antimicrobial activity of heme is signify-cantly impacted on the widely studied probiotic gram-positive *Lactobacillus plantarum*[56]. In addition, the heme diet not only damages the outer layer of IECs but also induces crypt depth deepening through mucolysis, causing cell proliferation by suppressing Wif1[56,57]. Given that the intestinal mucosal barrier is weakened and GNB is more prominent after heme exposure, the likelihood of LPS exposure to IECs should be raised. However, macrophage and neutrophil infiltrations are not observed after heme feeding for 14 days. Comparable to the histopathological finding, Toll-like receptor 4 (TLR4), a key

receptor that stimulates inflammatory response and is activated by LPS, is greatly downregulated[56,57]. Intriguingly, while the use of broad-spectrum antibiotics can considerably reduce the damage caused by heme on IECs, the recognition of heme-induced cell proliferation is solely detected with the presence of GM, and antibiotics have no influence on heme-induced cytotoxicity and lipid oxidation[57], signifying the importance of GM for heme-induced colon tumorigenesis.

Despite no correlation between heme-induced cell hyperproliferation and inflammation being indicated by the previous studies[56,57], there is evidence of myeloid cell infiltration and increased cytokine expressions in IECs following 21 days of dietary heme consumption[58]. Subsequent to cellular damage, neutrophil infiltration promotes cell proliferation via β-catenin signaling to maintain cellular homeostasis[59]. The number of cells with cyclooxygenase-2 (COX-2) expression, a major enzyme-producing inflammatory mediator, is augmented following the administration of heme for 21 days[58]. Prostaglandin E2 (PGE2), one of the products of the COX-2 pathway, activates phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)/Akt pathway signaling via E-type prostanoid receptor (EP) 2 and EP4 in LoVo cell line. GSK3β, one of the destruction complexes, is phosphorylated, resulting in the inhibition of β-catenin destruction. Subsequently, β-catenin accumulates in the cytoplasm and eventually migrates into the nucleus, enhancing cell proliferation[60]. Similarly, Wnt signaling is found to be activated by COX-2/PGE2 in glioblastoma[61]. Overall, prolonged heme consumption may lead to cellular hyperproliferation through a chronic inflammatory response involving COX-2/PGE2/PI3K-Akt/β-catenin pathway, which is not seen with short-term dietary heme intake.

It has been identified that chronic colitis plays a major role in the progression of CRC. The presence of TLR4 is not only abundant in IECs from human and murine-induced CRC cases[62,63], but the activation of TLR4 is also strongly related to crypt hyperproliferation[64]. The expression of both mucosal COX-2 and PGE2 is substantially lower in TLR4-knockout mice as compared to wild-type mice, which is consistent with the lower CRC tumor progression among TLR4-knockout mice[63]. Similar to TLR4 inhibition, the administration of antibiotics in mice also displays a favorable result in regards to chronic colitis-induced CRC tumorigenesis through the reduction of intestinal inflammation[62]. Following TLR4 activation by LPS, GSK3β is phosphorylated at Ser9 as a result of the PI3K/Akt signaling pathway. The phosphorylation of β-catenin at Ser552 and Ser675 is induced accordingly, stabilizing

β-catenin and facilitating its nuclear translocation. As expected, the Wnt target gene cyclin-D1 is significantly upregulated, corresponding with the crypt hyperproliferation[64]. Interestingly, both *APC* wild-type and mutated colon epithelial cells demonstrate the LPS/TLR4-inducing β-catenin stabi-lization[64,65], the impairment of cell apoptosis is only observed in the *APC*-mutated cell. After TLR4 activation, phosphorylated nuclear factor kappa B (p-NF-κB) is promoted, resulting in increased β-catenin levels and subsequent cell proliferation. For controlling cell growth, the cytosolic β-catenin is abolished by a destruction complex, while the key apoptosis mediator caspase-3 is stimulated by elevated levels of p-NF-κB, leading to cell apoptosis. Conversely, excessive β-catenin in *APC*-mutated cells blocks p-NF-κB, and in turn, decreases the activation of caspase-3 and cell apoptosis[65]. Given that NF-κB activation is regulated by Akt and leads to an increase in COX-2 expression[66], it is likely that NF-κB signaling serves as the connection between LPS/TLR4/PI3K-Akt/β-catenin and COX-2/PGE2/PI3K-Akt/β-catenin signaling, and is a key modulator of cell survival[67].

Taken together, the initial effects of short-term heme consumption include the erosion of IECs, mucolysis, and gut dysbiosis. Although there is no inflammatory response during the short period of heme consumption, consuming heme for an extended period may result in uncontrolled cell destruction and an imbalance in GM, which can trigger a chronic inflammatory response and subsequent excessive cell growth. Aside from the COX-2/PGE2 /PI3K-Akt/β-catenin signaling, enhanced LPS from intestinal imbalance can activate the TLR4/PI3K-Akt/β-catenin pathway, thereby causing an excessive growth of IECs. NF-κB may be a link between these two signaling pathways. Lastly, cells with *APC* gene mutation demonstrate weakened caspase-3 activity following TLR4 stimulation, thus inhibiting cell apoptosis. The different responses of *APC*-mutated cell compared to the wild type are summarized in Table 2 and Figure 2.

Table 1. The potential effects of heme on colon cancer progression

Study types	Model	Heme concentration	Main outcomes	Underlying mechanisms
In vitro	CMT93[13]	Heme-rich fecal water from in vivo study (19 ± 7 μmol/L and 1097 ± 484 μmol/L) for 24 h	↑Cytotoxicity	N/A
	CMT93[41]	Heme-rich fecal water from in vivo study (49 μM) for 24 h	↑Cytotoxicity	N/A
	APC$^{+/+}$ colon epithelial cells[42]	Heme-rich fecal water from rat fed with 0.36 and 0.72 mmol/g hemoglobin for 24 h	↑Cytotoxicity, ↑apoptosis	↑caspase 3
	Wild-type colon epithelial cells[44]	Heme-rich fecal water from rat fed with 1% heme or 50% raw beef sirloin for 6 h and 24 h	↑Apoptosis	↑caspase-3/7 activation, ↑nuclear Nrf2, ↑HO-1
In vivo	Fischer 344 female rats with AOM-induced colon cancer[13]	Chicken (undetected heme), beef (0.6 μmol/g of heme), or black pudding (16 μmol/g of heme) at 600 g/kg meat of the total diet for 100 d	↑ACF	↑Fecal TBARS
	Fischer 344 female rats with DMH-induced colon cancer[41]	Beef (0.6 μmol/g of heme) at 60% meat of the total diet for 100 d	↑ACF	↑Fecal TBARS
	Fischer 344 female rats with AOM-induced colon cancer[42]	0.36 and 0.72 mmol/g hemoglobin for 100 d	N/A	↑Fecal TBARS, ↑Fecal HNE,
	C57Bl6/J male mice[45]	0.5 μmol/g heme for 14 days	Disrupt IEC surface, ↓Apoptosis, ↑cell proliferation, ↑cell cycle (G1/S, S and M phases)	↓Wif1, ↓Ihh, ↓Bmp2, ↓IL-15, ↑cyclins E1, A2 and B2, ↑survivin, ↑Xiap, ↑Ier3
	C57Bl6/J male mice[46]	0.2 μmol heme/g diet	↑Cytotoxicity, ↑cell proliferation	↑ MDA, ↑ROS

Superscripts indicate the reference; symbols (↑ and ↓) indicate increase and decrease in the obtained variables, respectively; h, hours; N/A, not available; Nrf2, Nuclear erythroid-related factor-2; HO-1, Heme oxygenase-1; d, days; AOM, Azoxymethane; ACF, Aberrant crypt foci; TBARS, Thiobarbituric acid reactive substances; DMH, 1,2-dimethylhydrazine; HNE, 4-hydroxynonenal; IEC, Intestinal epithelial cell; Wif1, Wnt inhibitory factor 1; Ihh, Indian hedgehog; Bmp2, Bone morphogenetic protein 2; IL-15, Interleukin -15; Xiap, X-linked inhibitor of apoptosis; Ier3, Immediate early response 3; MDA, Malondialdehyde; ROS, reactive oxygen species.

Table 2. The different responses of *APC*-mutated cell compared to the wild type

Cell line	Inducer	Main outcomes	Underlying mechanisms
APC$^{+/-}$ colon epithelial cells[42]	Heme-rich fecal water from rat fed with hemoglobin	↓Cytotoxicity, ↓apoptosis	↓caspase 3
APC-mutated colon epithelial cells[44]	Heme-rich fecal water from rat fed with 1% heme or 50% raw beef sirloin	↓Apoptosis	↓caspase-3/7 activation, ↑↑nuclear Nrf2, ↓HO-1
Lentivirus-mediated shRNA knockdown of *APC* expression-HCT116[57]	LPS	↓Apoptosis	↓p-β-catenin, ↓p-NF-κB, and ↓caspase-3
Tumor-bearing female nude mice model of shAPC-HCT116[57]	Intratumoral injection LPS	↑Tumor volume and weight	N/A

Superscripts indicate the reference; symbols (↑ and ↓) indicate increase and decrease in the obtained variables, respectively; h, hours; d, days; Nrf2, Nuclear erythroid-related factor-2; HO-1, Heme oxygenase-1; LPS, Lipopolysaccharide; NF-κB, nuclear factor kappa B; N/A, not available.

Future perspective

Although the role of *APC* gene mutation in colon carcinogenesis is well-established, there is limited research on the effects of dietary factors in *APC*-mutated models. The importance of bioactive food components in preventing CRC has been receiving increasing attention over the past two decades[68]. Several studies have focused on *APC* gene mutation and the Wnt pathway. For instance, curcumin, the main active ingredient in turmeric, decreases the progression of colonic polyps in *Apc*$^{Min/+}$ mice fed with a high-fat diet by enhancing apoptosis and DNA repair[69]. Additionally, quercetin, a plant compound belonging to the flavonoid group, has been found to be effective in controlling colon carcinogenesis caused by DMH through the Wnt/β-catenin pathway[70]. Besides, rats fed with quercetin show upregulation of Nrf2, protecting against DMH-induced colon carcinogenesis[71]. However, it has been discovered that the overexpression of Nrf2 contributes to the progression of CRC[72,73], in line with the observation of increased nuclear Nrf2 levels in colon epithelial cells with *APC* mutations. As a result, evaluating the effects of bioactive food components on Nrf2 activity in heme iron-driven colon tumorigenesis, especially those with *APC*

mutations, is of considerable significance. It is necessary to ensure the safety of using these components in heme iron-related colon cancer cases. Taking advantage of this understanding, this insight can be used to design a specific dietary guideline to prevent colon cancer in individuals with *APC* gene mutation or those with first-degree relatives diagnosed with FAP.

According to the role of GM on colon carcinogenesis, exploring the potential effects of gut dysbiosis and metabolites produced by GM on neoplasia can be a focus of future research. Lastly, *Apc*$^{Min/+}$ mice are primarily used for in vivo studies, and their cells can also be utilized as *APC*-mutated cells for in vitro studies. Nevertheless, *Apc*$^{Min/+}$ mice are more likely to develop small intestine cancer, and fewer colon tumors are found. This point is the main limitation of using *Apc*$^{Min/+}$ mice to resemble cases of CRC in humans[74]. Additional investigations should be conducted on organoids derived from adenomatous polyps to validate their effects in humans[75].

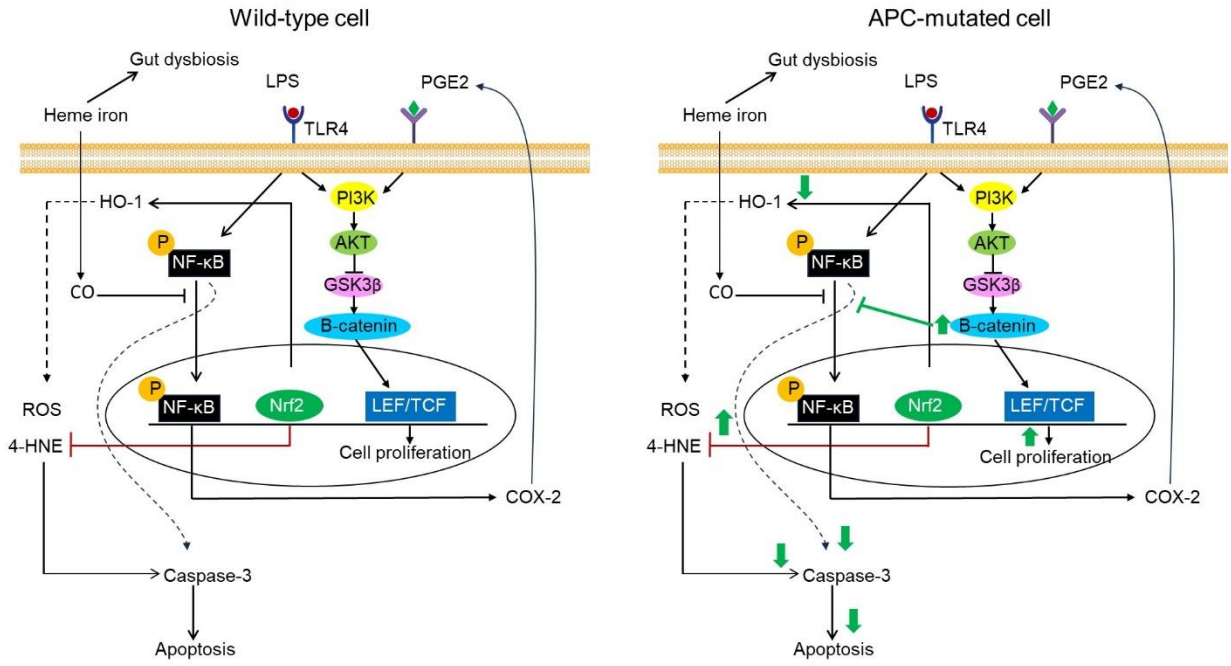

Figure 2. The different responses of *APC*-mutated cells on heme iron exposure compared to the wild-type

Conclusion

The potential mechanisms of heme-related colon carcinogenesis are as follows: 1) DNA damage from hydrogen peroxide formation; 2) Compensatory hyperproliferation of colon epithelial cells by down-regulated Wif1; 3) Increased cell proliferation due to chronic inflammation caused by gut dysbiosis, involving COX-2/PGE2/ PI3K-Akt/β-catenin and LPS/ TLR4/PI3K-Akt/β-catenin pathways; and 4) Selectively promoting *APC*-mutated cells survival through lower toxicity of 4-HNE, augmentation of the antioxidant defense mechanism, and inhibition of p-NF-κB, causing reduced caspase-3 activity and decreased cell apoptosis. The latter mechanism should be given more attention to decrease the chance of colon cancer developing in individuals with an *APC* gene mutation or those with first-degree relatives who have been diagnosed with FAP. Limiting heme iron-rich foods and considering dietary supplements that provide antioxidant, anti-inflammatory, and gut microbiota modulation benefits should be taken into consideration.

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None

Conflicts of Interest

The authors declare that there is no conflict of interest.

Abbreviation

4-HNE, 4-hydroxynonenal

ACF, aberrant crypt foci

AOM, azoxymethane

APC, Adenomatous polyposis coli

CIN, chromosomal instability

CK1, casein kinase 1

COX-2, cyclooxygenase-2

CRC, colorectal cancer

DMH, 1,2-dimethylhydrazine

EP, E-type prostanoid receptor

FAP, familial adenomatous polyposis

GNB, gram-negative bacteria

GM, gut microbiota

GPB, gram-positive bacteria

GSK3β, glycogen synthase kinase 3β

HO-1, heme oxygenase-1

IEC, intestinal epithelial cell

ISCs, intestinal stem cells

KRAS, Kirsten rat sarcoma viral oncogene homolog

LPS, lipopolysaccharide

MDA, malondialdehyde

Nrf2, nuclear factor erythroid 2-related factor 2

PGE2, prostaglandin E2

PI3K, phosphatidylinositol-4,5-bisphosphate 3-kinase

PUFAs, polyunsaturated fatty acids

p-NF-κB, phosphorylated nuclear factor kappa B

ROS, reactive oxygen species

TBARS, thiobarbituric acid reactive substances

TLR4, Toll-like receptor 4

Ser, serine

Wif1, Wnt inhibitory factor 1

ZnPP, Zn(II) protoporphyrin IX

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