The role of heme iron molecules derived from red and processed meat in the pathogenesis of colorectal carcinoma

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Highlights:

- Three main mechanisms for heme induced colorectal carcinogenesis are discussed
- Heme cytotoxicity by accelerating programmed cell death and epithelial hyperplasia
- Heme induce lipid peroxidation and form DNA adducts and mutate APC gene
- Heme catalyses N-nitroso-compound production and genetic mutations
- Balanced diet reduces risk of heme induced colorectal carcinogenesis

Abstract

Emerging evidence that heme iron in red meat is a risk factor for colorectal carcinogenesis is a topic that has received recent scrutiny. This review aims to summarise the mechanism of colorectal carcinogenesis by heme contained in red and processed meat. Heme iron can induce cytotoxicity by 'cytotoxic heme factor' and promote surface epithelial cell apoptosis and compensatory epithelial hyperplasia. Heme, induces peroxidation of lipids, leading to free radical formation and generation of DNA adducts in colorectal epithelial cells. In addition, heme catalyses the formation of N-nitroso-compounds, which in turn results in the initiation of colorectal carcinogenesis. Emerging data suggest that intestinal dysbiosis can promote carcinogenic properties of heme. Heme induces multiple genetic alterations by regulating *WNT* signalling pathway and causing mutations in major colon cancer genes such as *APC*, *TP53* and *KRAS*. However, a balanced diet containing green vegetables, olive oil and calcium may reduce the carcinogenic effects of heme.

Keywords:

Colorectal cancer; carcinogenesis; heme; red meat; processed meat; cytotoxicity; gene mutation; *WNT*

1. Introduction

Meat a versatile food, is a source of high quality proteins, essential amino acids and many micronutrients including iron, selenium, zinc, vitamin B6, vitamin B12 and omega-3-polyunsaturated fatty acids (Celada et al., 2016). Red meat is defined as flesh from mammals (pork, beef, lamb, veal,etc.), with a higher percentage of red muscle fiber than white (Abid et al., 2014). Meat which is preserved and flavoured using methods such as salting, smoking, fermentation and curing is commonly referred to as processed meat (Bouvard et al., 2015). Previously, many epidemiological and experimental studies have demonstrated a strong association between red or processed meat consumption and colorectal carcinoma (CRC) (Bernstein et al., 2015; English et al., 2004; Sandhu et al., 2001; Smolinska and Paluszkiewicz, 2010) (Table 1). For instance, Sandhu et al. have confirmed that there is a 49% increase in the risk of developing CRC with daily consumption of 25g of processed meat and a 12-17% increased risk with daily consumption of 100g of red meat (Sandhu et al., 2001). Similarly, Smolinska and colleagues have shown carcinogenic effects of red meat in colon, following daily intakes of 50g or more (Smolinska and Paluszkiewicz, 2010). In addition to CRCs, high consumption of red and processed meat has also shown to be associated with increased incidence of many other carcinomas such as oesophageal, gastric,breast, pancreas and lung (Cross et al., 2011; Inoue-Choi et al., 2016; Lam et al., 2009; Taunk et al., 2016).Recently, following an assessment of over 800 studies across the world, the International Agency for Research on Cancer (IARC), a working group for cancer related studies of the World Health Organization (WHO), has confirmed that there is sufficient evidence for an association between (CRC) and consumption of processed meat (Bouvard et al., 2015).The IARC working group has classified

processed meat as 'carcinogenic to humans'/ Group 1 and red meat as 'probably carcinogenic to humans' / Group2A (Bouvard et al., 2015; Tasevska et al., 2011). However, the IARC group has concluded that there is insufficient evidence to prove a strong association between consumption of unprocessed red meat and carcinogenesis, since the effect of the other confounding dietary factors such as salt and fat, food preparation methods and various lifestyle factors were not explicitly considered in the studies reviewed (Bouvard et al., 2015). Despite this well-established relationship between processed meat and carcinogenesis in humans, the cellular and molecular mechanisms underlying the pathogenesis remains ambiguous.

Recent studies have proposed a number of probable mechanisms elucidating the association between red/processed meat and CRC. Some of the molecules in the red and processed meat including heme, N-nitroso compounds (NOCs), heterocyclic amines (HCAs), N-glycolylneuraminic acid (Neu5Gc) and polycyclicaromatic hydrocarbons (PAHs) have been proposed as carcinogenic mediators (Jeyakumar et al., 2017). Many studies have identified heme iron as the key molecule responsible for the pathogenesis of CRC following red/processed meat intake (Genkinger et al., 2012; Sesnik et al., 2001). However, the exact mechanism by which heme iron molecules in red/processed meat interact with colorectal cells is still vague and controversial.

In this study, we aimed to review the relevant literature on various cellular and molecular mechanisms involved in heme-induced colorectal carcinogenesis to better understand its pathophysiological implications.

2. Methods

Literature related to the roles of heme iron in human carcinogenesis from past 40 years was identified from the Pub Med database. The MeSH Terms used were 'red meat or processed meat', 'cancer or carcinogenesis' and 'heme'. Only full-text articles written in English, from 1977 to present date, were selected. The search resulted in a total of 114 studies, while only 87 studies were found to be directly relevant to the current review.

3. Discussion

Heme iron in red and processed meat appears to influence CRC initiation, differentiation and progression by a number of mechanisms. Heme iron and its metabolic byproducts interfere at different stages of this process to alter either the normal physiological pathways or trigger the colorectal carcinogenesis. The possible mechanisms by which heme drives colorectal carcinogenesis, are discussed below.

3.1 Heme and its absorption

Heme is a combination of ferrous iron and a heterocyclic macrocycle organic compound, porphyrin. The iron (Fe) atom in the heme molecule is located within its protoporphyrin ring (Figure 1). The molecule also has a large hydrophobic tetrapyrrole ring with two propionic acid side chains (Sesnik et al., 2001). It has three main variants, namely, *a*, *b* and *c*, of which heme *b* is the most abundant in mammals (Larsen et al., 2012). Mammals have a significant amount of heme *b* in hemoproteins such as myoglobin and haemoglobin. Heme *b* is bound to the

hemoproteins non-covalently, thereby allowing relatively easier dissociation from the hemoproteins, compared to heme *c* and heme *a*, releasing free heme (Park et al., 2006). Enteric absorption of heme in humans is believed to occur via two main mechanisms, namely, receptor mediated endocytosis of heme and direct transportation of heme by heme transporters HCP1 (Shayeghi et al., 2005) and FLVCR (Khan and Quigley, 2011) respectively.

Heme is absorbed mostly in the upper part of the small intestine. However, in individuals who consume large amounts of red/processed meat, all the ingested heme cannot be absorbed by the small intestine (Oostindjer et al., 2014). The unabsorbed heme reaches the large intestine and may remain there for a considerable time, especially if there is a longer transit time due to low fibre diets (Massey et al., 1988). Therefore, the large intestinal mucosa of regular red meat eaters is exposed to relatively high concentrations of heme over an extended period. Thus, the heme-induced carcinogenic effects are mostly evident in the large intestine, compared to the other regions of the gastrointestinal tract (Sesnik et al., 2001; Stoker, 1990).

Once absorbed, the degradation of heme is catalysed by the hemeoxygenase (HO) enzymes, either HO-1 or HO-2 producing Fe2+, biliverdin and carbon monoxide. HO-2 is the enzyme responsible for the catabolism of heme in normal physiological conditions (Gozzelino et al., 2010). However, in the presence of excess intracellular heme, expression of HO-1 isoform accelerates, thus increasing the excess heme breakdown rate (Kumar and Bandyopadhyay, 2005). This reaction, in turn, leads to the formation of labile Fe, which is later scavenged by ferritin molecules. Once all the HO-1 enzyme and ferritin molecules are saturated by extremely high doses of free heme, the cells accumulate free heme and labile Fe (Stoker, 1990) (Figure 2).

3.2 Heme induced cytotoxicity

Free heme, if present in excess amounts, has been found to exert significant cytotoxic effects on cells (Kumar and Bandyopadhyay, 2005; McIntosh and Leu, 2001). Heme concentrations above 100μM have shown to exert cytotoxic effects on human colonocytes in an in vitro study done on primary colonocytes and colonic tumour cells (Glei et al., 2006). The cytotoxic effect of heme appears to be mediated by a two-step process , which leads to increased membrane permeability and subsequent cell lysis (Schmitt et al., 1993). The hydrophobic properties of heme enable it to get inserted into the lipid layers of the plasma membrane and then it facilitates peroxidation of the plasma membrane lipids by promoting the formation of reactive oxygen species (ROS) (Light and Olson, 1990; Rose et al., 1985). At least 4 mechanisms have been proposed for heme-induced ROS production; 1- directly by the Fe molecule within heme via the Fenton reaction; 2- by free Fe molecule released from heme;3- by activation of signalling pathways to induce ROS generation enzymatically; 4- by converting hydroperoxides into free radicals (Dutra and Bozza, 2014). A study by Ijessennagger indicated that mice fed with high heme-containing diets showed acute oxidative stress on epithelial cells (within first two days) by the release of oxidative stress markers such as Vnn 1(Ijssennagger et al., 2012b).

Lipid peroxides formed by the action of ROS, covalently bind with the protoporphyrin ring of heme forming an extremely lipophilic molecule. This molecule, namely cytotoxic heme factor (CHF) is thought to mediate the changes in cell turnover and proliferation. In mice, after four days of heme ingestion, CHF has shown significant toxicity towards colonic epithelial surface cells by expressing high levels of cytotoxic stress markers such as Tis7, Nemo and necrosis inducer receptor interacting protein kinase-3 (Ijssennagger et al., 2013).

Free radicals generated by the action of heme, are capable of activating programmed cell death of colonic epithelial cells, via the activation of tumour necrosis factor (TNF) (Dutra and

Bozza, 2014). Further, ROS can activate sustained c-Jun N-terminal Kinase (JNK) signalling transduction pathways, which in turn induces the activation of effector caspases resulting in programmed cell death in colon cells (Kraaij et al., 2005) (Figure 2). Thus, apoptosis of damaged surface epithelial cells is accelerated by the free radicals formed by heme.

Heme induces increased crypt signalling to replace the surface epithelial cells which are being shed (Ijssennagger et al., 2012b). Further, heme accelerates hyperproliferation of stem cells in the crypt region, leading to hyperplasia. Downregulation of feedback inhibitors of cellular proliferation such as Wif1, Il-15, Ihh and Bmp2 by heme, may accelerate this process. (Ijssennagger et al., 2013; Ijssennagger et al., 2012b). Taken together, heme, being able to promote apoptosis of surface cells with accelerated hyperproliferation of crypt cells, may cause hyperplasia of the colonic epithelium, which may eventually lead to the development of colonic cancer (Figure 3).

3.3 Heme-induced lipid peroxidation and free radical formation

Heme iron is a catalyst for lipid peroxidation and free radical formation. Evidence suggests that these free radicals may be responsible for the cytotoxic effect of heme (Pierre et al., 2007). Muscle and other tissues of raw red meat contain high concentrations of oxymyoglobin and deoxymyoglobin, while the cooked and processed red meat contain proteins such as hemichromes and hemochromes. These proteins when digested, form amino acids, peptides and heme group molecules. The iron molecule present in heme reacts with nitrogen, sulphur and oxygen of amino acids and peptides. This coordinated heme and free heme are hypothesised to catalyse lipid peroxidation of polyunsaturated fatty acids (PUFA) in the cellular membranes of intestinal cells (Baron and Andersen, 2002; Gueraud et al., 2015). Heme-initiated lipid

peroxidation on the cell membrane leads to inactivation of membrane-bound enzymes and receptors, leading to the decreased fluidity of the membrane and increased nonspecific permeability to ions such as calcium (Kanner, 2007). Tolosano and Altruda found that the process of lipid peroxidation is in fact initiated in the stomach (Tolosano and Altruda, 2002). When unprocessed red meat comes in contact with human gastric acid, lipid hydroperoxide (LOOHs) is produced, which is then broken down to produce free radicals by the iron molecules released from heme (Kanner, 2007). Therefore, in the presence of abundant heme iron, LOOHs undergo the following reaction; LOOH+ Fe-ligand (heme) →LOOFe ligands→Lipid alkoxy radical + hemeoxyradical (Marnett, 1999). These ligands give rise to aldehyde products such as malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE). MDA is capable of interacting with DNA both in vitro and in vivo, forming adducts with deoxyadenosine, deoxyguanosine and deoxycytidine (Marnett, 1999), which can further induce genetic mutations in the intestinal epithelium (Niedernhofer et al., 2003; Yau, 1979). The major mutagenic adduct to DNA of MDA origin is M_1G, which is a pyrimidopurinone. M_1G is found in small amounts in the liver, white blood cells, pancreas and breast tissues of healthy human beings. However, in excess amounts, it may become the major endogenous mutagenic DNA adduct and can eventually lead to malignant phenotypical changes in cells (Marnett, 1999) (Figure 3).

3.4 Catalysis of N- nitroso compounds (NOC) formation by heme

N-nitroso compounds (NOC) are powerful carcinogenic substances that are detected in many types of food, including red and processed meat (Tricker and Preussmann, 1991). Further, NOCs are formed by colonic bacterial decarboxylation of amino acids due to the action of a nitrosating agent. Normal microbial flora of the colon was initially thought to be facilitating the production of NOCs by the release of bacterial nitrate reductase (Massey et al., 1988). As

opposed to this, Lunn and colleagues have noted that heme facilitates the formation of NOCs even in the upper gastrointestinal tract without any colonic microbial flora (Lunn et al., 2007). Therefore, it is likely that NOC substances can be ingested via food or can be formed in vivo both in the presence and absence of microbial flora.

Previous studies have suggested that heme catalyses the formation of NOCs in the gastrointestinal tract. Faecal excretion of apparent total N-nitroso-compounds (ATNCs) was increased in rats and mice fed with diets high in heme content (Mirvish, 1995). The follow-up studies have noted similar findings in humans. Excretion of ATNC with faeces was observed to be increased in humans fed with diets high in red meat (240g/day) compared to controls fed with none or little (60g/day) red meat (Hughes et al., 2001). Higher concentrations of NOC production is associated with increased amounts of red meat consumption and longer transit times due to low fiber diets (Bingham et al., 1996; Hughes et al., 2001).

NOC compounds such as nitrosamines, nitrosamides and nitrosoguanidines are alkylating agents that have the potential of generating mutations by G-A transition in *KRAS* gene in the colonic, gastric, bladder and oesophageal mucosa (Gilsing et al., 2013; Mirvish, 1995). *KRAS* mutations are reported in over half of CRCs and play a vital role in the oncogenic transformation of colorectal epithelial cells (Bos et al., 1987; Vogelstein et al., 1988). Further, nitrosylated compounds can react with colonic cell DNA and produce pro-mutagenic substances such as O^6-methylguanine and O^6-carboxymethylguanine. In addition, nitrosated glycine has been shown to induce mutations in *TP53,* a key tumour suppressor gene in the colorectum (Senthong et al., 2013).

Nitrosamines, when activated by the cytochrome P450 enzymes in the endoplasmic reticulum, form α hydroxynitrosamines and undergo spontaneous decomposition to produce

alkyldiazohydroxides. These compounds alkylate nucleophiles to produce diazoalkanes, which in turn can alkylate DNA bases especially at N7 and O6 of guanine and O4 of thiamine (Bastide et al., 2015). The O^6- Alkyl-guanines, induces G:C to A:T mutations by coupling with thiamine instead of cytosine, at the second base of codon 12 or 13 of *KRAS* genes and these mutations are considered as a preliminary step in colorectal carcinogenesis (Mirvish, 1995) (Figure 3).

3.5 Heme and intestinal microbial flora

Intestinal microbial flora contributes to the maintenance of various metabolic, immune and homeostatic functions of the normal intestinal epithelium. Any changes in the number, distribution or stability of the bacterial flora could trigger carcinogenesis, by causing chronic inflammation of the epithelium (Terzic et al., 2010). There is compelling evidence that dietary heme alters the normal bacterial flora especially by decreasing the number of gram-positive bacteria (Ijssennagger et al., 2012a). Gram-positive bacteria show selective susceptibility to cytotoxic faecal substances as opposed to gram-negative bacteria, which can resist the heme-induced cytotoxicity. This causes a decrease in the gram-positive bacteria in the gut and this, in turn, leads to an expansion of gram-negative colonies (Ijssennagger et al., 2012a) leading to a state of dysbiosis (microbial imbalance or maladaptation).

Intestinal bacteria are thought to induce hyperproliferation of epithelial cells via modulating oxidative cytotoxic stress and by influencing the mucosal barrier. These effects are heightened in the presence of heme in the colon (Ijssennagger et al., 2015). Oxidative stress induces the formation of lipid peroxides, which in the presence of heme, will lead to the production of CHF. This can later exert cytotoxic stress on colonic epithelial cells (Ijssennagger et al., 2015; Sesnik et al., 2001). Ijessennagger and colleagues demonstrated that diet of mice

when changed from a control diet to heme rich diet, exhibited lag time for the production of CHF and initiation of colonic epithelial hyperproliferation. This lag time is hypothesised to be a result of the time taken for colonic microbes to adapt to the changes in diet (Ijssennagger et al., 2013). Thus, the cytotoxic action of CHF on colonic epithelial cells appears to be facilitated by the colonic microbes.

Dysbiosis in the colon has shown to be linked with CRC (Constante et al., 2017; Louis et al., 2014; Nakatsu et al., 2015). Inflammatory bowel disease (IBD), a chronic inflammatory condition of the intestines, significantly increases the risk of CRC development (Broström et al., 1986; Greenstein et al., 1981; Lennard-Jones et al., 1990). Dysbiosis has been consistently found in patients with inflammatory bowel disease (IBD) (Frank et al., 2007; Manichanh et al., 2006; Peterson et al., 2008; Walters et al., 2014), suggesting a possible connection between dysbiosis and CRC. In addition, dietary heme has shown to cause dysbiosis and exacerbate the colitis and adenoma formation in mice (Constante et al., 2017). The faecal metagenomic functional contents of mice fed on a high heme-containing diet demonstrated similar changes to that of mice fed with colitis inducing agents (DSS), as opposed to mice on a control diet. High consumption of heme has led to the alteration of genes responsible for cell signalling, carbohydrate, lipid, vitamin, and cofactor metabolism. These changes were similar to those observed in patients with IBD (Constante et al., 2017).

Therefore, heme iron from red and processed meat may be one of the potential causes for the imbalance in the colonic microbial community, leading to chronic inflammation and possibly, the development of CRCs.

3.6 Heme induced genetic alterations in the colorectum

Evidence suggests that heme from red and processed meat can facilitate CRC via various genetic modifications (Fearon, 2011) (Figure 3). The normal epithelial architecture of the colon is maintained by a controlled and well-balanced shedding of surface epithelial cells and replacement by new cells from the crypts. This process is maintained by the regulation of multiple homeostatic signals especially by *WNT* signalling pathway (Lamprecht and Lipkin, 2002). Interestingly, the key genes involved in the *WNT* signalling pathway such as *WNT1*, *WNT11*, *PTPRT*, *WSIP1* and *TNF*, were reported to be up-regulated with high heme ingestion (Pierre et al., 2004). Further, there is experimental evidence that heme iron can cause G>A transition in adenomatous polyposis coli (APC) gene (Gilsing et al., 2013). APC is a tumour suppressor gene and around 80% of colonic tumours are found to harbour mutations in it. APC is also one of the main negative regulators of β catenin of *WNT* signalling pathway (Schneikert and Behrens, 2007). Due to the loss of APC action following mutation, β catenin accumulates within colonic epithelial cells and gets translocated to the nucleus and forms a complex with DNA binding factor TCF (T cell factor). This will further lead to activation of transcription factors such as MYC and D1, promoting uncontrolled cell proliferation of the colonic epithelial crypts (Schneikert and Behrens, 2007). Mucosal pentraxin (Mptx) is a diet modulated gene marker, which helps in maintaining the integrity and cell turnover of the colonic mucosal epithelium. Kraaiji and colleagues have reported downregulation of Mptx, due to the constant exposure to high concentrations of heme (Kraaij et al., 2005). This may also contribute to the loss of epithelial integrity and normal epithelial architecture of the colon.

Heme molecules derived from red/processed meat are also capable of inducing APC gene mutations by another mechanism. Heme can produce 4-HNE in the gastrointestinal tract as a result of lipid peroxidation (as discussed in 3.3). This compound was shown to be more cytotoxic

to wild-type, normal colonic epithelial cells than to APC mutated pre-neoplastic cells in mice (Baradat et al., 2011). Thereby, 4-HNE formed as a by-product of heme peroxidation can provide a survival advantage to APC mutated colonic cells rather than healthy colon cells. This, in turn, facilitates increased viability and proliferation of APC mutated cells compared to normal colonic cells.

TP53 is another tumour suppressor gene which is found to be mutated in most colon carcinomas (Russo et al., 2005). Heme catalyses NOC formation in the colon (Cross et al., 2003) and some NOCs have a direct alkylating effect and others can be metabolised to produce alkylating intermediates to initiate G>A transition in *TP53* and *KRAS* genes (Gilsing et al., 2013). In addition, both heme iron and NOCs can catalyse the formation of reactive oxygen species that may cause G>T conversion in *KRAS* and *APC* genes (Shibutani et al., 1991). These mutations can result in 'loss-of-function' of the *TP53* gene in the later stages of tumour development. Thus, it can be hypothesised that heme iron and its metabolic by-products will assist in colorectal carcinogenesis by inducing mutations in major tumour suppressor genes (*APC* and *TP53*) and oncogenes (*KRAS*). Further studies are required to confirm the observed associations between genetic mutations and heme intake via red/processed meat consumption.

3.7 Agents that reduce the carcinogenicity of heme

Previous studies have confirmed that cytotoxicity of heme is diminished with the addition of green vegetables to the diet in rats (de Vogel et al., 2005). Green vegetables contain a high amount of chlorophyll which is a structural analogue to heme, since it is a phytol-esterified magnesium porphyrin (de Vogel et al., 2005). Therefore, porphyrin contained in chlorophyll can compete with the porphyrin in heme to bind with lipid peroxides formed by the action of ROS.

Thus, chlorophyll can reduce the production of CHF, thereby decreasing the cytotoxicity and hyperproliferative effects of it. Further, chlorophyll can form a complex with heme, thereby blocking the site of covalent modification of heme (de Vogel et al., 2005). This process too can reduce the formation of heme related cytotoxic factor and thus its detrimental effects on the colonic epithelium.

In addition to chlorophyll, calcium may reduce the cytotoxic effects of heme due to their ability to precipitate heme molecules (Hallberg et al., 1993; Kraaij et al., 2005; Pierre et al., 2013). Previous studies have also noted that diets high in calcium in rats eliminate the carcinogenic properties exerted by heme. This is evident by reduced formation of pre-cancerous dysplastic lesions, aberrant crypt foci and mucin-depleted foci (Pierre et al., 2004; Pierre et al., 2003). Taken together, it can be hypothesised that calcium levels in the body might help in modulating heme-induced cell toxicity in the colonic epithelium thereby controlling colon cancer pathogenesis.

In addition, olive oil and antioxidants evidently reduce the cytotoxicity of heme in the intestinal epithelium (Pierre et al., 2003). Lee and colleagues have reported that consumption of horsemeat, despite being the 'most red meat', carries a low risk of CRC compared to pork or beef. This was suggested to be due to the presence of high concentrations of palmitoleic and alpha-linolenic acid in horse meat, which act as anti-carcinogenic agents (Lee et al., 2007). Therefore, diets high in calcium, olive oil, green vegetables and antioxidants could potentially eliminate the cytotoxic and carcinogenic effects of heme iron in red or processed meat products (de Vogel et al., 2005; Pierre et al., 2003).

3.8 Controversies of heme related carcinogenesis

Few epidemiological and experimental studies have indicated that heme is unlikely to be a potential culprit for carcinogenesis (Andersen et al., 2011; Tasevska et al., 2011). In a recent systematic review of many experimental studies on heme iron-induced colorectal carcinogenesis, it was concluded that most of the previous experimental studies had used a large amount of red/processed meat than which regularly consumed in human diet. Those experimental diets were deficient in protective compounds, which are usually present in regular human diets (Turner and Lioyd, 2017). Therefore, the validity of heme or red and processed meat associated colorectal carcinogenesis is still questionable.

On the other hand, 'white meat controversy' points to a major issue related to heme-mediated carcinogenesis in the colorectum, as the heme content of pork is not very high when compared to that of chicken, which is white meat. Lombardi-Boccia and colleagues indicated that the average heme iron content in cooked pork and chicken meat was 0.39±0.2mg/100g and 0.28±0.1mg/100g (of fresh meat) respectively, whereas cooked beef had a heme content of 2.63±0.5mg/100g (Lombardi-Boccia et al., 2002). Several epidemiological studies have proven that consumption of chicken is not associated with CRC, but in spite of being low in heme content, pork is usually identified as a potential culprit of CRC (English et al., 2004; Norat et al., 2005). Thus these findings suggest that heme may not be the sole causative factor of CRC associated with meat consumption.

In addition, a mammalian cell surface protein and a carcinogenic agent, N-glycolylneuraminic acid (Neu5Gc) is present abundantly in both pork and beef but low or absent in chicken. This suggests a non-heme mediated carcinogenic effect of red and processed meat consumption (Byres et al., 2008). Therefore further research is needed to unveil the role of different molecules present in red and processed meat in colorectal carcinogenesis.

4. Conclusion

In this review, we attempted to summarise the mechanisms (Figure 3) by which heme in red and processed meat induces colorectal carcinoma. Carcinogenesis of heme can be mediated by its ability to interfere and alter multiple molecular and genetic mechanisms in the colonic epithelium. Excess free intracellular heme causes overburdening of HO-1 and ferritin, leading to accumulation of free heme and labile iron within the colonic epithelial cells which can give rise to increased cell permeability, ROS production and subsequent cell lysis. Formation of CHF and ROS by heme may further accelerate surface epithelial cell apoptosis and crypt cell hyperplasia leading to CRC development. Further, heme causes peroxidation of lipids and NOC formation resulting in free radical release and triggering of genetic mutations leading to colorectal carcinogenesis. Emerging data also suggest that dysbiosis or microbial imbalance plays a major role in magnifying the heme-induced cytotoxic carcinogenic effects on colonic epithelium. In addition, heme induces multiple genetic alterations in the colon via regulating *WNT* signalling pathway and inducing mutations in key regulatory genes such as *APC, TP53* and *KRAS* genes in the colorectum. On the other hand, substances such as calcium, chlorophyll in green vegetables, antioxidants and olive oil can reduce the heme related carcinogenic effects in the colorectum. Therefore, a balanced diet containing all these components could potentially minimise the carcinogenic effect of heme iron in red and processed meat.

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Figure legends

Figure 1: Schematic representation of structure of Heme *b*

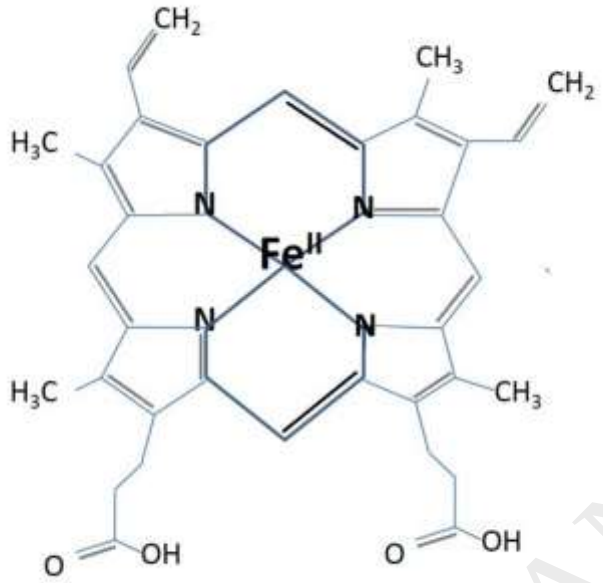


Figure 2: Mechanisms by which heme exerts its cytotoxic effects.

HO-1: Hemeoxygenase 1; JNK: c-Jun N-terminal kinase; ROS: reactive oxygen species; TNF: tumour necrosis factor

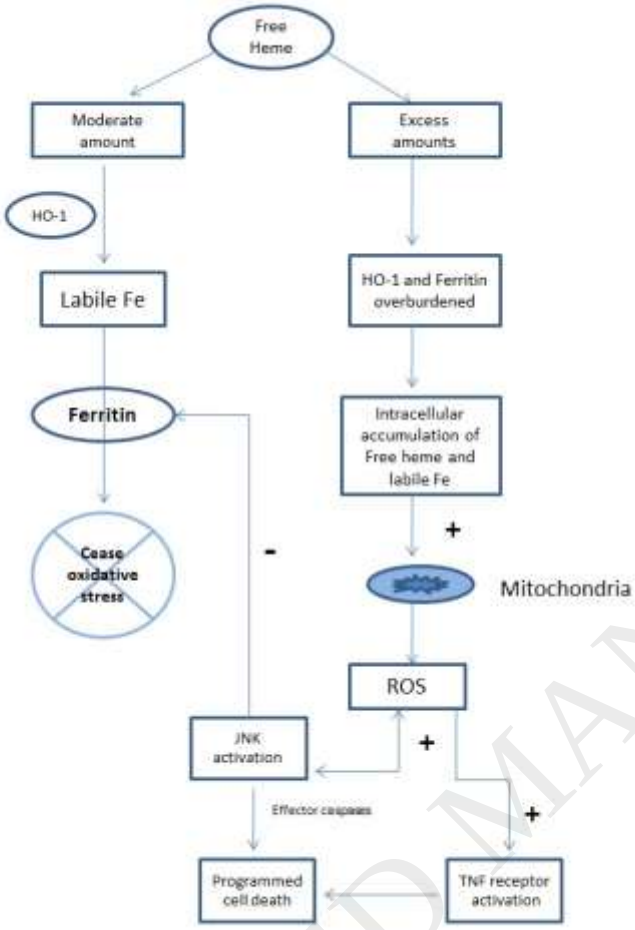


Figure 3: Mechanisms of Heme-induced carcinogenesis.

PUFA: polyunsaturated fatty acids; NOC: N- nitroso compounds; MDA: Malondialdehyde; HNE:4-hydroxynonenal; GIT:Gastrointestinal tract; APC: Adenomatous polyposis coli

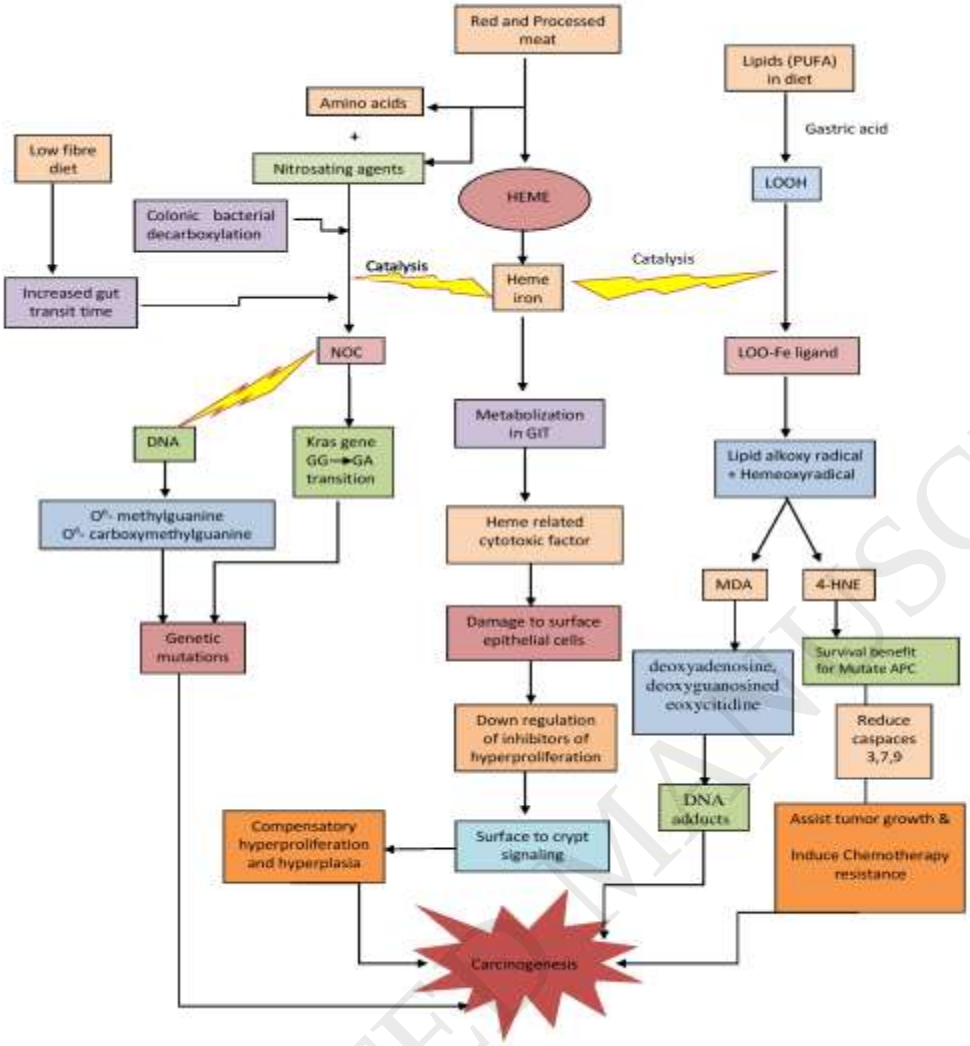
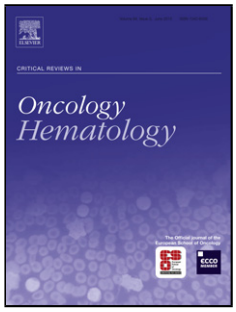


Table 1 Original research articles describing mechanisms of heme related carcinogenesis of colon/ rectum and their significant findings

Year	*First Author*	*Type of Study*	*Subject number*	*Human /other*	*Organ/ Tissue*	*Conclusions*
2015	Bastide NM(Bastide et al., 2015)	Experimental	125	Rats	Colon	Confirmed association between heme iron in red meat and colon cancer. Probable mechanism being heme iron lipid peroxidation
2015	Martin OC (Martin et al., 2015)	Experimental	62	Rats	Colon	Intestinal flora increases the lipid peroxidation by heme iron; thereby intestinal bacteria may have a role in colonic carcinogenesis of heme iron.
2012	Ferrucci LM (Ferrucci et al., 2012)	Prospective/ observational	17072	Human	Distal colon and rectum	Mostly meat related components are responsible for rectal CA. But dietary iron and iron from supplements show inverse association with distal CRC
2011	Andersen V(Andersen et al., 2011)	Cross-sectional	57053	Human	Colon, rectum	Hemeoxygenase1polymorphismis not associated with risk of CRC; heme iron is not an important factor determining CRC development
2010	Santarelli RL (Santarelli et al., 2010)	Experimental	140	Rats	Colon	Aerobically stored cured meat increased the number of pre-neoplastic lesions, indicating promotion of colonic carcinogenesis.
2006	Balder HF (Balder et al., 2006)	Prospective/ observational	120852	Human		Positive association between increased consumption of heme iron/ decreased consumption of chlorophyll with colorectal carcinogenesis in men
2004	Pierre F (Pierre et al., 2004)	Experimental	344	Rats	Colon	Red meat with high heme contents promotes colon carcinogenesis

CA: Carcinoma



Title: The role of heme iron molecules derived from red and processed meat in the pathogenesis of colorectal carcinoma

Authors: S.M.K. Gamage, Lakal Dissabandara, Alfred King-Yin Lam, Vinod Gopalan

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