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Influence of methyl donor nutrients as epigenetic regulators in colorectal cancer: A systematic review of observational studies

Epigenetic regulators in colorectal cancer

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Abstract

BACKGROUND

Dietary methyl donors might influence DNA methylation during carcinogenesis of colorectal cancer (CRC). However, whether the influence of methyl donor intake is modified by polymorphisms in such epigenetic regulators is still unclear.

AIM

We conducted a systematic review on this topic to improve the current understanding of the molecular basis of CRC.

METHODS

A literature search in the Medline database and manual reference screening were performed to identify observational studies published from inception to May 2022.

RESULTS

A total of fourteen case–control studies and five cohort studies were identified. These studies included information on dietary methyl donors, dietary components that potentially modulate the bioavailability of methyl groups, genetic variants of methyl metabolizing enzymes, and/or markers of CpG island methylator phenotype and/or microsatellite instability, and their possible interactions on CRC risk.

CONCLUSION

Several studies have suggested interactions between methylene tetrahydrofolate reductase polymorphisms, donor methyl-nutrients (such as folate) and alcohol on CRC risk. Moreover, vitamin B_6, niacin, and alcohol may affect CRC risk through not only genetic but also epigenetic regulation. Identification of specific mechanisms in these interactions associated with CRC may assist in developing targeted prevention strategies for individuals at the highest risk of developing CRC.

Core Tip: Dietary methyl donors might influence DNA methylation during the carcinogenesis of colorectal cancer (CRC). However, whether the influence of methyl donor intake is modified by polymorphisms in such epigenetic regulators is still unclear. We conducted a systematic review on this topic to improve the current understanding of the molecular basis of CRC. Several studies have suggested interactions between methylene tetrahydrofolate reductase polymorphisms, donor methyl nutrients (such as folate) and alcohol on CRC risk. Moreover, vitamin B_6, niacin, and alcohol may affect CRC risk through not only genetic but also epigenetic regulation.

INTRODUCTION

Colorectal cancer (CRC) is the third most frequent type of cancer and is responsible for the second highest mortality rate in cancer patients worldwide[1]. Although screening for early detection of CRC is effective to help decrease the trends in mortality rates, understanding daily life factors is also important to prevent this type of cancer[2]. The main factors which may help prevent CRC are those associated with diet, lifestyle, and prevention of metabolic diseases[3].

With regards to the dietary component, nutrients associated with one carbon (1C) metabolism (including folate, other B vitamins, methionine (Met), and choline) have been recognized as anticarcinogenic and chemotherapeutic agents in the 1C metabolic network[4]. Folate has shown to play a preventive role in CRC, probably because of its involvement in the processes of DNA methylation and synthesis[5]. Other nutrients,

such as Met and vitamins B_6 and B_{12}, which interact metabolically with folate in this process, may also influence the risk of CRC[6]. Moreover, in some of those studies, the observed inverse association between folate status and CRC risk was further modified by genetic polymorphisms of the enzymes involved in folate metabolism, most notably methylene tetrahydrofolate reductase (*MTHFR*).

A common C677T substitution in the *MTHFR* gene results in a protein with valine instead of alanine, yielding a more thermolabile enzyme with decreased activity[7]. Numerous studies have shown that this variant (TT) is associated with a decreased risk of CRC, but only when folate status is normal or high. *MTHFR* polymorphism is possibly the best-known gene polymorphism that switches from being a risk factor to a protective one depending on nutrient status. In any case, it is also important to evaluate the joint influence that other polymorphisms in genes involved in folate metabolism might exert. Thus, for example, Met synthase requires vitamin B_{12}, as methylcobalamin, as a cofactor. A variant in this gene, A2756G (in 5-methyltetrahydrofolate-homocysteine methyltransferase (*MTR*) gene), has been described and results in the substitution of aspartate by glycine. Some studies have shown that the MTR 2756 GG genotype is associated with a decreased risk of CRC; however, the association with diet is still unclear[8,9].

Another relevant example of a mutation in a polymorphism associated with CRC risk, but not consistent with nutrients, is the case of serine hydroxymethyltransferase, a pyridoxal phosphate (B_6)-dependent enzyme, in particular the polymorphism C1420T[10]. An additional relationship between 1C metabolism-related nutrients and CRC risk is related to its influence on DNA methylation. Thus, low folate status or intake is related to a decreasing methylation level[11,12], whereas colonic mucosal DNA methylation increased globally as a result of folate supplementation[13]. A sufficient intake of methyl donors may also prevent aberrant CpG island promoter hypermethylation. The promoter CpG island hypermethylation that characterizes the CpG island methylator phenotype (CIMP) in CRC is common[14]. Moreover,

polymorphisms in enzymes involved in folate metabolism may change the potential impact of methyl donor consumption on DNA methylation[15].

According to the molecular subtype, chromosomal instability, and microsatellite instability (MSI) represent the major pathways for CRC[16]. The inactivation of DNA mismatch repair (MMR) genes, which are responsible for correcting mismatched bases during DNA replication, results in MSI[17]. Microsatellites are short sequences that are dispersed across the genome and are likely to undergo MMR machinery-induced deletion or insertion. Defects in the MMR machinery are associated with CRC[18] and can be affected by epigenetic alterations and deregulation of methylation[19]. In this sense, the associations between methyl donor nutrient intake and CRC risk have been extensively studied, although evidence on their effect is limited[20]. However, whether they act as effect modifiers against a background of deficient DNA repair capacity is unknown.

Until now, there have been few studies on the influence of methyl group donors as epigenetic regulators in CRC[21]. In the present work, we reviewed previous studies that have investigated this matter to improve the current understanding of the molecular basis of CRC, which could contribute to a better design of future research and to better preventive nutritional management in this type of cancer.

MATERIALS AND METHODS

Search strategy

The search terms and search strategy were developed by two researchers. To guide this research, we formulated the following question as the starting point: What is the influence of methyl donor nutrients as epigenetic regulators in CRC? For this purpose, a systematic search in the Medline (through PubMed) database, Reference Citation Analysis (an artificial intelligence technology-based open multidisciplinary citation analysis database), and a manual reference screening were performed to identify observational studies published from inception to 20 May 2022. A search for relevant keywords and medical subject heading terms related to dietary methyl donors, dietary

components that potentially modulate the bioavailability of methyl groups, genetic variants of methyl-metabolizing enzymes, markers of CpG island methylator phenotype and/or microsatellite instability, in combination with keywords related to CRC events was conducted.

The search was amplified through citation chaining (forward and backward) of the included studies. Reference lists of all identified articles and other related review articles, systematic reviews, and meta-analyses were hand-searched for additional articles. The present search was developed according to the "PRISMA Statement" guidelines (see the PRISMA checklist in Supporting Information Table S1) (www.prisma-statement.org). For this review, a protocol was not prepared or registered. The search strategy is detailed as follows:

Colorectal neoplasms/exp

((colorectal* or rect* or anal* or anus or colon* or sigmoid) adj3 (cancer* or carcinoma or tumour* or tumor* or neoplas* or adenoma or adenocarcinoma)). Abstract (ab), keyword hearing word (kf), original title (ot), title (ti), text word (tw).

1 OR 2

(observational or case– control or cohort). Ab, kf, ot, ti, tw

(incidence or prevalence or risk or odds ratio or hazard ratio). Ab, kf, ot, ti, tw

(one-carbon metabolism-related nutrient* or dietary methyl donor* or alcohol). Ab, kf, ot, ti, tw

(gen* or SNP* or polymorphism* or methyl metabolizing enzyme* or diet–gene interaction* or hypermethylation* or microsatellite instability*). Ab, kf, ot, ti, tw

3 AND 4 AND 5 AND 6 AND 7

Review process and selection criteria

Two researchers independently screened the titles and abstracts of the articles to identify potentially relevant studies. Studies that passed the title/abstract review were retrieved for full-text review. The inclusion and exclusion criteria and the quality of the

study were assessed by two researchers with the use of a data extraction form especially designed for this study.

The inclusion criteria consisted of studies: (1) with an observational design (case–control or cohort); (2) that evaluated the exposure to at least one of the following dietary components (dietary nutrient intake and/or plasma levels): folate, other B vitamins, Met, choline, betaine, and/or alcohol; (3) that included genotyping analyses of methyl-metabolizing enzymes (*MTHFR, MTR*, methionine synthase reductase), DN methyltransferase 3 b, euchromatin histone methyltransferase 1 and 2, PR domain zinc finger protein 2; and/or (4) CIMP defined by promotor hypermethylation (calcium voltage-gated channel subunit a1 G, insulin-like growth factor 2, neurogenin1, runt-related transcription factor 3, suppressor of cytokine signalling 1), human *MutL* homolog (*MLH1*) hypermethylation; and/or (5) MSI using markers (such as mononucleotide microsatellites with quasi-monomorphic allele length distribution in healthy controls but unstable (Bat-26 and/or Bat-25), NR-21, NR-22, NR-24)); (6) in which the outcome of interest was CRC, colon, or rectal cancer (studies investigating benign adenomas or polyps were excluded); (7) that provided estimates of the adjusted odds ratios or relative risks or hazard ratios with 95% confidence intervals; (8) conducted in humans (≥ 18 y old); and (9) written in English or Spanish.

The following types of publications were excluded: (1) nonoriginal papers (reviews, commentaries, editorials, or letters); (2) meta-analysis studies; (3) off-topic studies; (4) studies lacking specific CRC data; (5) nonhuman research; (6) studies conducted in children, adolescents, or pregnant women; (7) duplicate publications; and (8) low-quality studies (Newcastle–Ottawa scale (NOS)[22] < 4 indicating insufficient study quality).

Study quality assessment

To evaluate the validity of the individual studies, two reviewers worked independently to determine the quality of the included studies based on the use of the NOS for case–control or cohort studies[22]. The maximum score was 9, and a high score (≥6)

indicated high methodological quality; however, given the lack of studies on the subject under study, it was agreed to select those that had a score equal to or greater than 4. A consensus was reached between the reviewers if there were any discrepancies.

Data extraction

The data extracted for each individual study included the following: name of the first author, study design, characteristics of the study population (age range or mean age, sex, country), dietary exposure, dietary assessment instrument used, outcomes (including cancer site), comparison, odds ratio or relative risk or hazard ratio (95%CI), adjusted variables, and NOS. For case–control studies, the following additional information was extracted: number of cases and number of controls. For cohort studies, the following additional information was extracted: number of participants at baseline, number of CRC cases, and length of follow-up. These variables were judged to be most relevant to the outcome studied. Where multiple estimates for the association of the same outcome were used, the one with the highest number of adjusted variables was extracted. Template data collection forms and data extracted from the included studies will be made available upon request from the corresponding author.

RESULTS

Figure 1 shows the PRISMA flow diagram summarizing the identification and selection of the relevant publications. A total of nineteen studies were included in this systematic review: fourteen case–control studies[23-36] and five cohort studies[9,37-40]. In total, the case–control studies included 7055 cases and 9032 controls. The cohort studies included 256,914 participants, with 1109 cases recorded during follow-up periods that ranged from 7.3 to 22 y. Amongst the case–control studies, nine articles were conducted in the United States, two in the United Kingdom, two in Korea and one in Portugal. With regard to the cohort studies, three were conducted in the Netherlands and two in the United States.

Tables 1 and 2 summarize the main characteristics and findings of the case−control and cohort studies, respectively, on the interactive effect between single-nucleotide polymorphisms in genes encoding methyl-metabolizing enzymes, dietary methyl donors and dietary components that potentially modulate the bioavailability of methyl groups on CRC risk. **Tables 3 and 4** show the effects of dietary methyl donors and dietary components that potentially modulate the bioavailability of methyl groups on CRC risk, according to SNPs in genes encoding methyl-metabolizing enzymes and/or mutations in oncogenes, CIMP and/or MSI, in the case−control studies and cohort studies, respectively. **Table 5** provides a summary of the results of the studies included in the systematic review.

DISCUSSION

This review summarizes previous studies that have investigated the influence of methyl donor nutrients as epigenetic regulators in CRC. The dietary components that showed a higher association with CRC risk were folate and alcohol. Thus, high folate intake was considered a protective factor, while high alcohol consumption proved to be a risk factor. Several studies have investigated the association between methyl donor nutrients and/or methyl antagonists (*e.g.*, alcohol) and *MTHFR* polymorphisms and have reported significant interactions[23,24,26,29,36]. In one of those case−control studies, those with the *MTHFR* 677 TT genotype, who consume low folate diets, had a greater chance of developing CRC than people with the CC or CT genotype [24]. Two other case−control studies reported that *MTHFR* 677 TT carriers with high (above mean) or adequate folate intake had a low risk of CRC[26,29].

Two case−control studies found that alcohol consumption increased CRC risk among *MTHFR* 677 TT carriers[23,26]. The decreased *MTHFR* enzyme activity among those who carried the T allele, and consumed low methyl donor nutrients and large amounts of alcohol can be utilised to explain the increased CRC risk[25,30]. These dietary habits may alter folate metabolism, especially in people with folate deficiencies[41]. In contrast, several studies have not found an association between *MTHFR*

polymorphisms and either folate intake or alcohol intake and CRC risk[25,27]. It has been hypothesized that the differences in folate status among various populations may have influenced the contradictory results on the contribution of *MTHFR* genetic variants in CRC[25]. In addition to *MTHFR* polymorphisms, de Vogel *et al.* [9] also investigated the modifying effects of polymorphisms of methionine synthase reductase, *MTR*, DN methyltransferase 3 b, euchromatin histone methyltransferase 1 and 2, and PR domain zinc finger protein 2 but found no interactions, although some *P-trends* were significant.

Regarding the methylation abnormalities of genes, de Vogel *et al.*[37] found that high vitamin B_6 intake was associated with an increased CRC risk caused by hypermethylation of the *hMLH1* promoter among men. Therefore, these authors suggest that vitamin B_6 may have had a tumour-promoting effect by increasing promoter methylation. However, the intake of folate, vitamin B_2, Met and alcohol was not associated with the risk of tumours showing *hMLH1* hypermethylation. In any case, other studies showed inverse associations between vitamin B_6 intake and CRC risk, both when the intake level was higher[6] and when it was similar[42] to that in the study of de Vogel *et al.* [37]. Therefore, further attention should be given to this association in future studies.

Another interesting finding in this review is the inverse association between dietary folate and Met and B-Raf proto-oncogene mutations among men[37]. A previous study showed that folate may increase the risk of tumours harbouring truncating adenomatous polyposis coli mutations in men[43]. Apparently, relatively high folate intake may confer a growth advantage to mutated tumours independent of the type of mutation. Nevertheless, the occurrence of MSI does not seem to be sensitive to methyl donor intake or to that of alcohol[36-37,44].

In one of the case–control studies that we analysed, an interaction was observed between a high- or low-risk diet and *MTHFR* 1298A>C (but not 677C>T) with regard to CIMP status[32]. This result suggests that a genetic polymorphism at *MTHFR* 1298A>C interacts with the diet (with a low folate and Met intake and a high alcohol

consumption) increasing the risk of highly CpG-methylated colon tumours. S-adenosylmethionine binds as an allosteric inhibitor in the *MTHFR* regulatory region, which is where the 1298A>C variant is found. This gives some justification for the stronger associations between *MTHFR* 1298A>C and CIMP rather than the 677C>T SNP, which has an opposite effect on the stability of the enzyme. Due to their high linkage disequilibrium, the *MTHFR* 677C>T and 1298A>C polymorphisms should not be viewed separately. van Engeland *et al.*[40] also discovered that people diagnosed with CRC, with low intake of folate and high consumption of alcohol, had a greater prevalence of promoter hypermethylation; however, the difference was not statistically significant due to limited power. Therefore, it was proposed that stratification for functionally significant SNPs in the genes encoding folate metabolism enzymes could strengthen the observed effect of folate deficiency on promoter methylation[40].

Additionally, Curtin *et al.*'s study[32] found an interaction between alcohol consumption and *MTHFR* 1298A>C in association with CIMP status. Relative to the AA genotype in non-drinkers, the *MTHFR* 1298 AA genotype was linked to a higher risk of CIMP+ in drinkers. In a previous study by Slattery *et al.*[35], in which associations between CIMP status and alcohol use were assessed without taking into regard to genotype, no association was observed. These results imply that the activity of the 1C-metabolism enzyme may alter the risk associated with alcohol in determining the CIMP status of colon cancer. In any case, to date, few published studies have evaluated, CIMP in CRC for possible relationships with 1C-metabolism SNPs[32,45,46]. Moreover, these data raise the possibility that more investigation is required to clarify the function of genetic SNPs in relation to CIMP status and the promoter hypermethylation of particular genes.

In one case–control study[36], an association between long-term alcohol consumption and increased likelihood of having a CIMP-low or B-Raf proto-oncogene-mutated tumour was observed. Among those with unstable tumours, they observed that alcohol was more likely to be associated with CIMP-low rather than CIMP-high tumours. A previous study of these same authors showed that alcohol increased the risk for MSI+

tumours in general[35]. Therefore, these findings suggest that the increased risk of MSI associated with alcohol is limited to those tumours that are unmethylated rather than methylated.

Finally, regarding the interaction between MMR SNPs and methyl donor nutrient intake on CRC based on MSI status, in a case–control study[34], a strong inverse association was observed for *hMSH3* AG or GG carriers with a high intake of niacin, particularly among patients with CC and microsatellite stability or proficient MMR status. However, to ascertain the processes behind this association with CRC risk, the precise roles of this SNP must be identified.

The importance of interactions between modifiable factors, such as methyl donor nutrients and CRC, is suggested by evidence that colorectal carcinogenesis is generated by numerous molecular pathways in parallel with MSI status, which is caused by a defect in the MMR machinery. In this sense, it is worth remembering the article mentioned by de Vogel *et al.*[37], in which it was reported that high consumption of vitamin B_6 was linked to an increased risk of sporadic CRC with *hMLH1* hypermethylation, indicating that vitamin B_6 affects CRC risk through both genetic and epigenetic mechanisms. Based on the findings of Kim *et al.*[34], it may be possible to explain the interactions between dietary methyl donor nutrients, such as niacin, and *hMSH3* genetic variants as predictors of CRC risk.

DNA methylation and microRNA expression levels may be regulated by the MMR machinery's epigenetic relationships with methyl groups[48]. Through the methylation of CpG islands in the promoter region, 1C metabolism mediated by methyl donor nutrients can change how the DNA MMR system is activated. A adequate DNA MMR system with methyl groups may control the equilibrium between the repair and accumulation of short repeat sequences, preventing extensive DNA damage that supports colorectal carcinogenesis.

This systematic review has several strengths: (1) cohort and case–control studies were identified through a systematic search; (2) a quantitative NOS scale was used to assess the quality of the studies; and (3) most studies (twelve of nineteen) used a

validated questionnaire to assess dietary intake. To our knowledge, this is the first systematic review regarding the influence of methyl donor nutrients as epigenetic regulators in CRC.

The limitations of this review include the following: (1) case–control studies, which are susceptible to recall and selection bias, made up the bulk of the research. However, most studies were based on community controls; thus, they might be a good representation of the frequency of genetic variants or of dietary habits of the overall population; (2) the heterogeneous nature of studies, including the study population characteristics, sample size, study design, and follow-up periods; (3) potential residual confounding because of the observational nature of the studies included or the possibility that not all the studies were adjusted for important nutrient variables; (4) some of the dietary assessments were self-reported, which may affect the reliability of the reported intakes, although the use of validated questionnaires in most studies could reduce this bias; (5) some studies had a relatively limited sample size or effect size, which made it difficult for them to detect the interactions between genetic and nutritional information, which could explain, in part, the lack of results with a statistically significant level; and (6) despite the fact that some research have found differential interactions based on the CRC subtype[34], the majority of studies lacked stratified analysis. Considering these limitations, the conclusions from these researchers should be taken carefully. Consequently, it is difficult for this review to explain the gene-diet interactions and their effects on the development of CRC.

CONCLUSION

In this systematic review of observational studies, some interactions between *MTHFR* polymorphisms, donor methyl nutrients (such as folate) and alcohol on CRC risk are suggested. Moreover, some studies show that vitamin B_6, niacin and alcohol may affect CRC risk through not only genetic but also epigenetic regulation. In any case, this review was not able to clarify which mechanisms underlie the influence of methyl donor nutrients on DNA methylation, as well as the efficacy of methyl uptake,

transportation, and the final involvement in methyl-related gene expression. Further prospective studies with large samples and long follow-up periods, as well as clinical trials that take into account the long latency period of CRC, are needed to clarify the influence of methyl group donors as epigenetic regulators, with particular emphasis on differences in CRC subsite-specific risk. Such studies may provide valuable insight into the biological mechanisms with the goal of identifying at-risk subpopulations and promoting primary prevention of CRC.

ARTICLE HIGHLIGHTS

Research background

Colorectal cancer (CRC) is the third most frequent cancer and is responsible for the second-highest mortality rate in cancer patients worldwide. The main factors which may help prevent CRC are those associated with diet, lifestyle, and prevention of metabolic diseases. With regards to the dietary component, one-carbon metabolism-related nutrients have been considered anticarcinogenic and chemotherapeutic agents in the one-carbon metabolic network. However, it is still unclear whether the influence of methyl donor intake is modified by polymorphisms in these epigenetic regulators.

Research motivation

Although screening for early detection of CRC is effective to help decrease the trends in mortality rates, understanding daily life factors is also important to prevent this type of cancer. A better understanding of the molecular basis of CRC could contribute to a better design of future research and better preventive nutritional management in this type of cancer.

Research objectives

In the present work, we reviewed previous studies that have investigated this matter to improve the current understanding of the molecular basis of CRC.

Research methods

A literature search in the Medline database, Reference Citation Analysis (an artificial intelligence technology-based open multidisciplinary citation analysis database), and manual reference screening were performed to identify observational studies published from inception to May 2022. A search for relevant keywords and medical subject heading terms related to dietary methyl donors, dietary components that potentially modulate the bioavailability of methyl groups, genetic variants of methyl-metabolizing enzymes, markers of CpG island methylator phenotype and/or microsatellite instability, in combination with keywords related to CRC events was conducted. The present search was developed according to the "PRISMA Statement" guidelines. To evaluate the validity of the individual studies, two reviewers worked independently to determine the quality of the included studies based on the use of the Newcastle Ottawa scale for case control or cohort studies.

Research results

A total of fourteen case control studies and five cohort studies were identified. In total, the case control studies included 7055 cases and 9032 controls. The cohort studies included 256,914 participants, with 1109 cases recorded during follow-up periods that ranged from 7.3 to 22 y. The dietary components that showed a higher association with CRC risk were folate and alcohol. Thus, high folate intake was considered a protective factor, while high alcohol consumption proved to be a risk factor. Several studies have investigated the association between methyl donor nutrients and/or methyl antagonists (*e.g.*, alcohol) and methylene tetrahydrofolate reductase *(MTHFR)* polymorphisms and have reported significant interactions. In one of those case control studies, those with the *MTHFR* 677 TT genotype, who consume low folate diets, had a greater chance of developing CRC than people with the CC or CT genotype. Two other case control studies reported that *MTHFR* 677 TT carriers with high (above mean) or adequate folate intake had a low risk of CRC.

Research conclusions

In this systematic review of observational studies, some interactions between *MTHFR* polymorphisms, donor methyl nutrients (such as folate), and alcohol on CRC risk are suggested. Moreover, some studies show that vitamin B6, niacin, and alcohol may affect CRC risk through not only genetic but also epigenetic regulation.

Research perspectives

This review was not able to clarify which mechanisms underlie the influence of methyl donor nutrients on DNA methylation, as well as the efficacy of methyl uptake, transportation, and the final involvement in methyl-related gene expression. Further prospective studies with large samples and long follow-up periods, as well as clinical trials that consider the long latency period of CRC, are needed to clarify the influence of methyl group donors as epigenetic regulators, with particular emphasis on differences in CRC subsite specific risk.

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