Nutrigenetics and colon cancer

Abstract
Colon cancer is the third most common type of cancer and the second leading cause of cancer-related death in the western world. Many epidemiological studies have shown that lifestyle and intake of various food components influence the colon carcinogenesis. Novel concepts such as nutrigenomics and nutrigenetics which study the interaction between diet and genes with common goal to optimize health through personalization of diet, provide approaches to elucidate the complex relationship between nutrients and genetic polymorphisms involved in various diseases like colon cancer. Nutrigenetics of colon cancer is based on correlation between folate metabolism and polymorphisms in the genes encoding crucial enzymes in folate metabolism pathway. The folate pathway influences aberrant pyrimidine and purine synthesis as well as the global DNA hypomethylation and hypermethylation of tumor suppressor genes such as APC, hMLH1, p16 and E-cadherin in colon cancer. In addition, polymorphisms in the MTHFR, TYMS, MTR, MTRR, RFC, DHFR and other genes involved in the folate pathway have been shown to influence colon carcinogenesis. The central role of the folate pathway in the cell has made it a target for a variety of chemotherapeutics. Polymorphisms in the genes encoding crucial enzymes in the folate pathway might have an important role in the pharmacogenetics and nutrigenetics of colon cancer.

INTRODUCTION
Colon cancer is one of the most common types of cancer and one of the leading causes of death in the western world, so it is important to elucidate the influence of various factors on this widespread type of cancer. It has been observed that environmental and lifestyle factors are very important in the etiology of colon cancer. Numerous studies have shown that meat (1), fat (2), fibre and fruit and vegetable consumption (3) are associated with different development rates of colon cancer. The specific component of food which may protect against colon cancer is folate, a water-soluble vitamin B9. Its name was derived from the Greek word 'folium' which means 'leaf' since it is commonly found in fresh fruit and vegetables. There are numerous studies linking low folate intakes with colon carcinogenesis as well as with the increased risk for cardiovascular disease, neural tube defects, adverse pregnancy outcomes and neuropsychiatric disorders. On the other hand, there are numerous evidence for a protective effect of folate supplementation. This effect on neural tube defects (4) was considered to be sufficient for U.S. Food and Drug Administration (FDA) to issue a regulation in 1998 requiring that all flour and uncooked cereal grain products in the USA have to be fortified with folic acid (140µg/100g) (5, 6). Accordingly, many studies have shown high folate intake to be associated with the
prevention of colon cancer. In prospective cohort study performed on 88,756 women from the Nurses’ Health Study, Giovannucci et al. (7) showed that the long term (over 15 years) use of supplemental folate may substantially reduce the risk of colon cancer for up to 75%. These and other epidemiology studies have undoubtedly suggested that folate deficiency increases whereas folate supplementation decreases the risk of colon cancer (8, 9).

**Personalized medicine and nutrigenetics**

Most genetic variations, such as single nucleotide polymorphisms (SNPs), insertions, deletions or repeats have been determined by sequencing genes encoding enzymes related to the disease of interest (10). With sequencing of the whole human genome, the knowledge about genetic variations has increased and yielded novel concepts such as nutritional genomics which includes the fields of nutrigenomics and nutrigenetics (11). Nutrigenomics is the science that examines the response of individuals to food compounds using modern molecular biology technologies (e.g. genomics, transcriptomics, proteomics, metabolomics). The long-term aim of nutrigenomics is to understand how whole body responds to real foods using an integrated approach termed 'systems biology' (www.nugo.org). Nutrigenetics, on other hand, is based on understanding the gene-based differences in response to dietary components. Nutrigenetics of colon cancer is based on the correlation between folate metabolism and polymorphisms in the genes encoding crucial enzymes in the folate metabolism pathway (11, 12, 13).

**Folate and colorectal carcinogenesis**

In the cell, folate functions as a donor of one-carbon units and is essential for DNA, RNA and histone methylation as well as for nucleotide synthesis. Consequently, low folate status is associated with aberrant DNA methylation which includes global DNA hypomethylation as well as hypermethylation of CpG promoter sites of tumor suppressor genes such as APC and hMLH1 in colon cancer (14). APC tumor suppressor gene is inactivated in at least 90% patients with FAP (familial adenomatous polyposis) due to germ-line mutations. FAP is an autosomal dominant disease in which affected individuals develop over a thousand benign colorectal adenomas some of which inevitably progress into malignant carcinomas. Biallelic inactivation of the gene in familial adenomatous polyposis and most sporadic colorectal tumors promotes tumorigenesis (15). Inactivation of APC tumor suppressor gene may occur through multiple mechanisms, including allelic loss, gene mutation or by methylation of CpG promoter sites. APC promoter hypermethylation can complete APC loss of function in cases without apparent APC genetic alteration and, as a second hit according to Knudson’s two-hit hypothesis for tumor suppressor gene inactivation, in tumors with a one hit in one allele. Hypermethylation of promoter, like coding region mutations of APC appears with equal frequency in both preinvasive and invasive colon neoplasia which indicate that methylation plays an important role in the colorectal tumorigenesis (16).

According to these findings, it has been observed that MMR (Mismatch Repair) deficiency in sporadic colorectal cancer is frequently the result of epigenetic silencing of the hMLH1 gene by promoter hypermethylation (17). Such altered pattern of methylation which causes transcriptional loss has also been detected in other tumor suppressor genes such as p16 and E-cadherin, confirming the role of methylation in malignant progression (18, 19).

In plasma, folate is present in the form of 5-methyltetrahydrofolate (5-Methyl THF) which is a cofactor and methyl donor for the remethylation of homocysteine to methionine. The reaction is catalyzed by the enzyme methionine synthase (MTR) which uses cobalamin (vitamin B12) as a cofactor and by the enzyme methionine synthase reductase (MTRR). Methionine, an essential amino acid is then converted to S-adenosyl methionine (SAM) which functions as an universal methyl donor in the cell. Aberrant folate status results in promoter-specific hypermethylation of certain tumor suppressor genes (14, 20), as well as in global DNA hypomethylation, both characteristic to colon carcinogenesis. Several studies have shown that dietary supplementation can lead to changes in DNA methylation. In a randomized controlled trial of patients with colorectal adenoma, Pufulete et al. (21) have reported that supplementation with 400 μg folic acid per day for 10 weeks increased the global DNA methylation in both leukocytes (+31%) and colonic mucosa (+25%), which suggests that DNA hypomethylation can be reversed by physiological intakes of folic acid.

**Gene polymorphisms in folate pathway**

Almost a hundred genes are involved either directly or indirectly in folate metabolism (Figure1), and certain single nucleotide polymorphisms (SNPs) that are associated with colon cancer have been identified (13). It has been hypothesized that genetic polymorphisms in folate-metabolizing enzymes affect global DNA methylation and changes in the availability of nucleotides for DNA synthesis and repair (22).

The intracellular form of folate, 5,10-methylenetetrahydrofolate (5,10-Methylene THF) acts as a cofactor in the reductive methylation of 2'-deoxyuridine 5'-monophosphate (dUMP) to 2'-deoxythymidine 5'-monophosphate (dTMP) by thymidylate synthase (TS), which is a crucial reaction in the formation of thymidine in the cell. When folates are deficient in the cell, uracil can be misincorporated in DNA and during the repair by the uracil-glycosylase, chromosomal damage as instabilities in the form of breaks and translocation can be induced (23, 24). Both aberrant DNA methylation pattern as well as DNA damage are common in many types of cancer and accordingly in colon cancer. 5,10-Methylene THF is converted to 5-Methyl THF by methylenetetrahydrofolate reductase (MTHFR) encoded by the gene positioned at 1p36.3. The C>T SNP in position 677 yields a thermolabile enzyme that has been associated with in-
increased plasma homocysteine and lowered circulating plasma folate levels, so that an increase in colon cancer risk could be expected. This has been confirmed by several studies (25, 26, 27) but some studies (28, 29, 30) have reported the contrary. The MTHFR 677TT variant was associated with the 20–50% decreased risk of colon cancer largely in the presence of increased intakes of folate and other B-vitamins (29, 31). In addition, the risk of colorectal adenoma can be increased when there is low intake of nutrients involved in one-carbon metabolism (10, 32). Thus, when folate nutrition is adequate, the risk of adenoma or colon cancer is reduced. The inverse associations observed with the reduced MTHFR activity (MTHFR 677TT genotype) may be associated with accumulation of substrate for MTHFR, 5,10-Methylene THF, since it acts as a methyl donor in the reaction of conversion of dUMP to dTMP by thymidylate synthase (TS) (33). The promoter of gene for thymidylate synthase (TYMS) contains a variable number tandem repeat (VNTR) of 28bp which is associated with the altered expression of the protein. Individuals homozygous for the triple 28bp repeat (3R) have been shown to have up to 4-fold higher expression level of the TS protein compared to double repeat (2R) (34). Ulrich et al. (35) have shown in their study that individuals who showed low MTHFR activity (MTHFR 677TT genotype) and low TS expression (TSER 2R/2R genotype) were unexpectedly the group at the lowest risk of colorectal adenoma. The same author suggested that the availability of 5,10-Methylene THF for a third pathway, purine synthesis, may be another mechanism linking folate to colorectal carcinogenesis (12).

Many other genes in folate pathway have been investigated for polymorphisms that may be related to colon carcinogenesis. Methionine synthase (MTR) catalyzes the methylation of homocysteine to methionine by using cobalamin (vitamin B12) as a cofactor. During that reaction MTR may become deactivated but the enzyme methionine synthase reductase (MTRR) can reactivate it. Methionine synthase gene (MTR) contains the A-G polymorphism at bp 2756 which causes an aminoacid substitution from aspartic acid to glycine at codon 919 (D919G) (36). The A666G polymorphism in the methionine synthase reductase gene (MTRR) results in the substitution of isoleucine with methionine at codon 22 (37). Since reduced folate carrier (RFC) seems to be the main enzyme for 5-MeTHF transport into cells, its polymorphism 80G>A could have influence on folate status (38). A 19 bp deletion in the intron 1 of the dihydrofolate reductase (DHFR) gene was shown to remove a potential Sp1 transcription binding site which affects the transcription of the DHFR gene (39). Polymorphisms in the MTHFR, TYMS, MTR, MTRR, RFC, DHFR and other genes involved in the folate pathway have been shown to influence the colon carcinogenesis via methylation reactions on DNA, RNA, histones and other proteins, as well as nucleotide synthesis in the cell (10, 25, 28–30).

CONCLUSION

Folate supplementation can have protective effect against colon cancer when given prior to the formation of early lesions. Once preneoplastic lesions are formed, folate enhances tumor growth due to its role in the rapidly dividing cells that need it for the DNA synthesis. This central role of folate in the cell has made it a noteworthy target for the group of chemotherapeutic agents anti-folates which include 5-FU and methotrexate (MTX) that are commonly used in the treatment of colon cancer. It is clear that folate and certain SNPs in the folate pathway may have a crucial role in the nutrigenetics and pharmacogenetics of colon cancer.
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