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Diet, the Gut Microbiome, and Epigenetics

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Abstract

Increasingly, the gut microbiome is implicated in the etiology of cancer, not only as an infectious agent, but also by altering exposure to dietary compounds that influence disease risk. While the composition and metabolism of the gut microbiome is influenced by diet, the gut microbiome can also modify dietary exposures in ways that are beneficial or detrimental to the human host. The colonic bacteria metabolize macronutrients, either as specialists or in consortia of bacteria, in a variety of diverse metabolic pathways. Microbial metabolites of diet can also be epigenetic activators of gene expression that may influence cancer risk in humans. Epigenetic involves heritable changes in gene expression via post translational and post transcriptional modifications. Microbial metabolites can influence epigenetics by altering the pool of compounds used for modification or by directly inhibiting enzymes involved in epigenetic pathways. Colonic epithelium is immediately exposed to these metabolites, although some metabolites are also found in systemic circulation. In this review, we discuss the role of the gut microbiome in dietary metabolism and how microbial metabolites may influence gene expression linked to colon cancer risk.

Keywords

Gut microbiome; bacteria; diet; epigenetics; cancer risk

Diet can influence epigenetic changes associated with disease and may modify gene expression patterns through epigenetic mechanisms. (reviewed in[1, 2]) Epigenetic mechanisms that affect the expression of target genes include histone modifications, DNA methylation, and non-coding RNAs. The microbial metabolism of diet also produces compounds that alter epigenetics either by 1) altering pools of substrates used for modification such as methylation or 2) by generating other compounds that alter the activity of enzymes involved in epigenetic modification. These alterations persist from one cell division to the next, so even though there is no alteration in the gene sequence, epigenetic alterations are heritable. Infectious pathways, the genotoxic effects of microbial metabolites, and pathogens involved in epigenetics that affect host health are addressed elsewhere.[3, 4]

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Here, we present below examples of the role of the microbiome in dietary metabolism, an overview of eukaryotic epigenetic mechanisms, and microbially-derived metabolites that are relevant to eukaryotic epigenetic changes in gene expression that may be linked to colon cancer. In particular, we focus on the following microbial metabolites: the short chain fatty acids (SCFA), isothiocyanates, lipids, and select polyphenols.

Diet shapes the microbiome

Diet helps shape the composition of the intestinal microbiota. Differences in the distribution of bacteria are associated with dietary patterns and this influences host exposure to microbial metabolites. De Filippo et al. showed that the gut microbiome of children living in Burkina Faso was significantly different than European children of the same age.[5] The microbiome of the children from Burkina Faso was more varied, produced more butyrate, and had more Prevotella than Bacteroides. The children in Burkina Faso consumed a diet high in fiber and non-animal protein whereas the European children consumed a diet higher in refined carbohydrates and animal protein. Similar trends were seen in other studies that compared residents in non-industrialized and industrialized societies.[6, 7] Although these observational studies lay the ground work for the connection between diet and the microbiome in human populations, factors other than diet may influence the composition and function of the microbiome in these different habitats.[8]

Dietary intervention studies provide a controlled setting in which to study how the composition and function of the microbiome changes in response to diet. These studies show that the gut microbiome often responds quickly to changes in diet.[9-13] Of particular interest, dietary interventions also alter the exposure of the host to microbial metabolites although these changes are often transient.[14-18] For example, in a randomized, controlled cross-over designed dietary intervention, Costabile et al found that bacterial groups and plasma levels of bacterial phenolic metabolites were altered depending upon the type of fiber ingested in two, three week periods.[18] However, the microbial metabolites only increased in one of the treatments suggesting that diet is a complex human exposure that is further complicated by microbial metabolism and the composition of the microbiome.

Epigenetics

Epigenetics refers to heritable changes in gene activity that are not caused by changes in the DNA sequence.[19] Regulation of gene expression can occur through post translation modification of the amino acids in histone proteins, by methylation of DNA, and at the post-transcriptional level by miRNAs.[20]

Histone proteins are involved in tightly packing chromosomes into chromatin in the nucleus. Modification of histone proteins loosens the chromatin, making it more available for transcription. Post-translational modifications of the N-termini of histone 3 or histone 4, occur by acetylation, methylation, ubiquitination, phosphorylation, or sumoylation. The enzymes involved in these reactions include: histone acetyltransferases (HATs) which adds acetyl groups; histone deacetylases (HDAC), which removes them; histone methyltransferases (MHTs), which add methyl groups to lysine and/or arginine groups; and histone demethyltransferases (DMHTs), which removes them. HATs catalyze the addition

of acetyl groups to the lysine residues of histones that activates normally repressed genes. HDACs are responsible for the removal of these groups and results in gene repression.[21, 22] Methylation of lysines results in either activation or deactivation of transcription whereas methylation of arginine residues activates transcription. In cancer cells, patterns of histone modifications and methylation differ from normal cells. Hypermethylation of genes involved in pathways identified as the hallmarks of cancer: cell cycle regulation, DNA repair, apoptosis, hormone response, carcinogen metabolism, angiogenesis and metastases are found in several human cancers [2, 23-26]

Methylation of DNA in the coding region of a gene, especially the conversion of cytosine to 5-methylcytosine, leads to CpG dinucleotides that affect expression of target genes. DNA methylation patterns change in response to environmental factors through DNA methyltransferases (DNMT1, DNMT2 DNMT3A, and DNMT3B) in the presence of S-adenoysl-L-methionine.[27, 28] Hypermethylation of these CpG dinucleotides or CpG islands, if they are in the promoter regions of a gene, result in transcriptional gene silencing and gene inactivation.[20] Methylation has been linked to many diseases, including cancer.[29-32]

MicroRNAs (miRNA) are small, non-coding RNAs, which inhibit gene expression at the posttranscriptional level. miRNAs are generated by a complex protein system involving transcription of miRNA genes by RNA polymerase II and processing of the pre-miRNA by Drosha and Dicer ribonucleases.[33] Mature miRNA are part of an active RNA-induced silencing complex (RISC). miRNAs regulate post-transcriptional expression by either imperfect base-pairing to mRNA or by affecting mRNA stability. Each miRNA controls several genes in related pathways. Aberrant miRNA expression affects signaling pathways associated with the initiation and progression of carcinogenesis.[34]

The impact of bacterial metabolism of dietary fiber on epigenetics

Meta-analysis of prospective cohort and nested case-control studies have shown an inverse relationship between dietary fiber intake and risk of colon cancer, although these trends tend to be more variable in prospective cohort studies due to confounding variables.[35] Increased fiber intake reduces the exposure of gut epithelial cells to toxicants by increasing stool bulk which dilutes fecal carcinogens and decreases transit time. High-fiber foods contain an array of complex phytochemicals that are metabolized by the gut microbiome to short chain fatty acids, isothiocyanates, and polyphenolic derivatives that interact with human gut epithelial cells and may modify epigenetic control of gene expression.

Microbial metabolism of dietary fiber to SCFA

The main types of dietary carbohydrate that escape digestion in the small intestine are resistant starches, non-starch polysaccharides, and oligosaccharides primarily from plants. In westernized populations, approximately 40 g/day of carbohydrates enter the large intestine and undergo microbial metabolism.[36] There is a gradient in SCFA production from the proximal (70-140 mmol) to the distal gut (20-70 mmol) largely based on availability of substrate and SCFAs are inversely associated with increased pH in the distal colon. [36] The predominant short chain fatty acids from fermentation are acetate, butyrate, and propionate in a ratio of 3:1:1, although formate, caproate, and lactate are also formed.[37]

The fluxes through these pools vary by fiber source and microbial pathways dictated by microbial community composition of the lumen. While acetate is a dominant end product of glycolysis, cross-feeding between groups of bacteria occurs and can influence SCFA pools.[38] Butyrate forming bacteria condense acetate from butyrl CoA and external pools of acetate.[39, 40] Use of external pools of acetate varies among bacterial species and may alter the amount of acetate and butyrate available to the host. For example, Duncan et al. showed that while *F. prausnitizii* and *Roseburia spp* derived about 85% of butyrate carbon from external pools of acetate, *Coporcoccus spp.* only derived 28%.[41] In addition, different sources of carbohydrates produce different amounts of butyrate ranging from 56% with pectin to 90% for xylan. Propionate is also formed via microbial fermentation of carbohydrates. After formation of pyruvate, depending upon the microbial composition, propionate is formed via the succinate or the more minor acrylate pathways from carbohydrates that reach the colon.[42] While butyrate supplies the majority of energy to colonic epithelial cells primarily through beta-oxidation, concentrations of SCFA in the colon are also high enough to influence regulation of colon epithelial gene expression.[43-45]

The role of butyrate in cancer prevention is the result of differences in the underlying physiology of normal or tumor cells.[46] Butyrate has differential effects on normal colon epithelial cells depending upon its concentration and the metabolic state of the cell. Butyrate is present in high concentrations in the lumen and is transported into eukaryotic gut epithelial cells.[47] In the colon crypts, there is a decreasing concentration gradient of butyrate from the lumen to the bottom of the crypt. In the cells at the bottom of the crypt, normal cell growth is supported by beta-oxidation of butyrate in the mitochondria and little butyrate accumulates in the nucleus.[48] There is increased cell proliferation via increased energetics. Normal cells near the lumen experience higher levels of butyrate which accumulates in the nucleus and inhibits HDAC. Cell proliferation is inhibited, apoptosis is induced, and the cells are exfoliated into the lumen. In normal homeostasis, butyrate plays a role in promoting cell turnover of the colonic epithelium. In contrast, metabolism in cancer cells is dominated by aerobic glycolysis which uses glucose over butyrate as the growth substrate. Butyrate can then accumulate in the nucleus where it functions as an HDAC inhibitor and inhibits cell proliferation and induces apoptosis.

While inhibition of HDACs is a common mechanism to inhibit cancer growth, the effect of SCFA on the prevention of colorectal cancer has been inconsistent. This may be due to differences in response among eukaryotic cell lines or effects of other SCFA. For example, acetate, propionate, and caproate have been shown to be inactive as HDAC inhibitors in HT-29 cells,[49-51] although the effect on gene expression is not clear in Caco-2 cells.[52]

Microbial metabolism of glucosinolates to isothiocyanates

Glucosinolates are converted into isothiocyanates (ITC) by either the plant myrosinases, or bacterially produced thioglucosidases. Cooking cruciferous vegetables deactivates the plant myrosinases, and given that most cruciferous vegetables consumed by humans are cooked, gut bacteria play a critical role in converting glucosinolates to ITC. Previous studies have shown that certain species of bacteria, such as *Escherichia coli, Bacteroides thetaiotaomicron, Enterococcus faecalis, Enterococcus faecium, Peptostreptococcus sp.* and

Bifidobacterium sp., isolated from the human gut or feces can convert glucosinolates into ITCs and other derivatives.[53-55] Controlled feeding studies in humans have shown significant inter-individual differences in urinary ITC excretion after participants consumed the same amount of cruciferous vegetables that had been either heated or microwaved prior to consumption to remove the plant myrosinase activity.[56-58] Similar effects have been found in studies with rats.[59, 60] This suggests inter-individual differences exist in the activity or composition of the intestinal bacteria involved in ITC formation. In support of this hypothesis, we showed recently that the fecal bacteria from individuals who excrete higher amounts of ITC in their urine after a standard meal of cooked broccoli metabolize more glucoraphanin *in-vitro*.[61]

Studies suggest that the exposure to ITCs *in-vivo* may well translate to prevention or reduction in tumor growth through the effects on DNA methylation, histone modification, and miRNA. Sulforaphane (SFN), an ITC, prevents carcinogen or genetically induced colon cancer in rodent models. Recent studies have shown that sulforaphane is an HDAC inhibitor and leads to an increase in global and local histone acetylation.[62, 63] Similar to butyrate, SFN causes p21 upregulation and cell cycle arrest. Further studies in wild-type and APC Min/+ mice showed that a single dose of SFN reduced HDAC activity and increase histone acetylation in colonic mucosa.[63] Additionally, SFN has been identified as a DNA demethylating agent in breast cancer cell lines although this has not been verified in colon tissue.[64]

Dietary polyphenols

Polyphenols represent a wide variety of phytochemicals that are divided into several classes according to their chemical structures. They include phenolic acids (hydroxybenzoic acids and hydroxycinnamic acids), anthocyanins, flavonoids (flavanols, flavonols, flavones, flavonones, and isoflavones), stilbenes, lignans, and curcuminoids. These compounds undergo extensive microbial transformations in the colon and also undergo enterohepatic circulation which alters influence host exposure to these compounds and their metabolites. While the dietary polyphenolic compounds have been associated with inducing epigenetic mechanisms, many of the microbial metabolites of polyphenols have yet to be tested even though metabolomics studies identify these compounds in systemic circulation. We present below evidence of the role metabolites of epigallocatechin-3-gallate in green tea and ellagitannins in relation to epigenetic modifications exists.[65]

EGCG

Epigallocatechin-3-gallate (EGCG) is a polyphenol found in green tea that has been reported to impact human health including anti-oxidative, blood cholesterol and sugar level lowering, and cancer preventive activities. However, epidemiologic studies show inconsistent results when evaluating green tea consumption in relation to colon cancer risk.[66-70] This is in part due to differences in tea consumption habits and failure to account for other factors, such as alcohol consumption or tobacco smoke, which may modify the effect of green tea consumption on health.[71, 72] A recent study found that green tea consumed at least three

times per week for more than 6 months was associated with reduced risk of colorectal cancer by 23% in non-smokers.[67]

Pharmacokinetic studies show that tea catechins do not appear to accumulate in systemic circulation but do undergo rapid absorption and elimination in humans.[73] During this period, they undergo extensive biotransformation by both Phase II enzymes and gut microbial metabolism. The metabolites/catabolites identified include glucuronides and sulfated conjugates of EGCG indicative of Phase II metabolisms, as well as, methylated catechins, ring fission products (like valerolactone), and phenolic products that are indicative of gut microbial transformations. Chow et al showed, in pharmacokinetic studies, that while green tea catechins (EGCG) were present in plasma after a single-dose, the concentration is lower than the amounts of biological activity shown in *in vitro* studies.[73] In contrast, the biotransformed compounds were present in higher amounts than the parent compound, suggesting that metabolites/catabolites should be considered in the biological effects of these polyphenols.

Polyphenols have exhibited differential selection for different species in the microbial community by either acting as an antibiotic or a prebiotic which may influence human exposure to microbial metabolites of polyphenols. Recently, van Dorsten et al showed, in a simulated gut bioreactor, that the type and distribution of microbial metabolites of polyphenols changed as a function of dosing strategy.[74] For example, valerolactones, one of the initial fission products of microbial metabolism of catechins, disappeared with continuous dosing. In parallel, Kemperman et al found that there were dramatic shifts in the composition of the microbiome with continual polyphenol dosing, although it is difficult to directly link the disappearance of valerolactones to specific bacteria.[75] These studies suggest that diet may alter the microbiome, which in turn alters dietary exposure and down-stream host response.

Green tea polyphenols, specifically EGCG, alter gene expression by influencing DNA methylation patterns and/or histone modification. Several studies have shown that EGCG inhibits both bacterial and eukaryotic DNMT and alters DNA methylation patterns.[76, 77] This mechanism is mediated by altering the availability of methyl groups that are used to methylate catechol groups on polyphenols by catecohol-O-methyltransferase (EC 2.1.16). Additionally, the microbial metabolites of EGCG, gallic acid (GA) and epigallocatechin (EGC) influence epigenetic gene expression by acting as HAT inhibitors[78-82] although not as strongly as EGCG.[82]

Ellagitannins

Ellagitannins are polyphenols that are found in fruits, such as pomegranate, raspberries, strawberries, blackberries, and nuts, such as walnuts and almonds. Ingested ellagitannins are hydrolyzed in the stomach and small intestine to ellagic acid. The gut microbiome metabolizes ellagitannins to urolithins by removal of one of the lactone rings and subsequent dehydroxylation in the colon. Enterohepatic circulation of urolithins and ellagic acids alters host exposure to these compounds, and there is large between person variation in urolithin production.[83] Recent studies suggest that bacteria from the *C. coccoides* group and

Actinobacteria are involved in the production of urolithins.[83, 84] Specifically, urolithin D was produced followed by urolithin C and urolithin B, and was associated with an increase in *Bifidobacteria spp* and *Lactobacillus spp*.

Ellagitannins have strong antioxidant, radical scavenging, anti-viral, anti-microbial, anti-mutagenic, anti-inflammatory, anti-tumor promoting, and immunomodulatory properties.[85] Ellagitannins inhibit proliferation and induce apoptosis of cancer cells through modulation of transcription factors and signaling pathways.[85] Ellagitannin treatment of a liver cancer cell line showed increased expression of miRNAs associated with cell differentiation and proliferation in a dose and time dependent manner.[86] Metabolites of ellagitannins, ellagic acid and the bacterial metabolites, urolithin B and C, also act as epigenetic modulators. At physiologic concentrations, ellagic acid and urolithins reduced HAT activity in a tumor necrosis factor (TNF) stimulated monocyte cell line[82] suggesting a plausible epigenetic mechanism mediated through gut microbial metabolites that is consistent with the observations that ellagitannins are an anti-inflammatory component of diet.

Dietary fats

The majority of dietary fats, such as triacylglycerol, saturated and unsaturated fatty acids, and sterols, are absorbed in the small intestine. However, recent studies suggest that 7% of ingested fat is excreted in stool and is likely metabolized by the gut microbiota.[87] In addition, bile acids are synthesized in the liver from cholesterol, conjugated with methionine or glycine. Bile is secreted from the gall bladder into the small intestine to help emulsify fats during digestion. Most of the bile acids (95%) are absorbed in the ileum and delivered back to the liver. A small amount is delivered to the colon and undergoes anaerobic microbial metabolism to secondary bile acids deoxycholate (DCA), lithocholate (LCA), and ursodeoxycholate (UDCA). Listed below are two examples of microbial metabolism of fats that may influence epigenetics.

Polyunsaturated Fatty Acids

Fish oil from cold water fish and plants provides dietary sources of ω 3 long chain polyunsaturated fatty acids (3ω LC-PUFA). Epidemiologic studies have shown that these fats are protective against colon and prostate cancer although there are conflicting results. [88] Prospective studies showed either no effect, increased risk, or reduced risk.[89-93] Differences in these outcomes have been attributed to sex differences, cancer stage, site of the disease, and other confounding factors. Case-control studies and experimental studies which focused on a defined dietary intake support the hypothesis of CRC risk reduction by 3ω LC-PUFA. A reduction in histone lysine methylation by 3ω LC-PUFA has been shown to down-regulate genes in cancer cell lines .[94] Other studies have shown that the type of 3ω LC-PUFA is important in miRNA mediated gene expression. Azoxymethane (AOM) tumor induction in rats fed either fish (3ω LC-PUFA) or corn oil (6ω LC-PUFA) showed that the fish oil intervention produced fewer tumors and the lowest number of differentially express miRNAs.[95] The anaerobic bacteria, *Roseburia*, *Bifidobacteria*, and *Lactobacillus*, found in the distal gut, metabolize 3ω LC-PUFA from dietary intake to conjugated linolenic (CLnA) acids.[96, 97] Although 3ω LC-PUFA are not produced by bacteria, the availability of 3ω LC-

PUFA may be influenced by microbial metabolism and may, in part, explain some of the inter-individual variation in the effects of 3ω LC-PUFA on cancer.

Ursodeoxycholate (UDCA)

Colon cancer is associated with a high-fat diet in western populations and rates in non-western populations are increasing as a more western, non-traditional diet is adopted.[98, 99] Recent studies in mice suggest that the gut microbiome influences the size and composition of the bile acid pool throughout the enterohepatic system and exposure may influence cancer risk.[100] For example, *R. gnavus* can convert 7-oxo-lithocholic acid to UDCA.[101] A study of the microbiome in colorectal and healthy subjects found a 63% increase in the amount of UDCA in stools of healthy individuals correlated with *Ruminococcus sp.*[102] Secondary bile acids, deoxychloate (DCA), lithodeoxycholate (LDA), and ursodeoxycholate (UDCA) increase in response to high fat diets and are increased in populations with higher incidence of colorectal cancers. In particular, there was a positive association with DCA and colorectal adenomas, a precursor to colorectal cancer.[103-109] In contrast, UDCA has been shown in animal and *in vitro* studies to reduce the risk of cancer dysplasia and cancer development[110, 111] and, in a phase III clinical trial, UDCAs reduced the degree of dysplasia in colonic polyps.,[112] Recent mechanistic studies suggest that, in contrast to butyrate, UDCA induced cell differentiation and senescence in colon cancer cells via histone hypoacetylation.[113]

Summary and Future Directions

Transformation of dietary compounds by the gut microbiome results in additional environmental exposures that may influence epigenetic mechanisms of gene expression. We presented examples of anaerobic metabolism by gut bacteria that is dynamic and responsive to ingested substrates from myriad dietary sources. Functional genes measured in the gut microbiome suggest that there are basic metabolic pathways that are conserved across all healthy individuals[114, 115] which have the core capacity to generate compounds that influence epigenetic pathways of gene expression.[109, 110] While colonic epithelium may have immediate exposure to microbial metabolites, many microbial products are absorbed into systemic circulation and may alter gene expression in regions distal to the gut. Integration of dietary intake, measurements of the gut microbiome, and epigenome markers in multi-generational human population studies are needed to understand the influence of these environmental factors on human health.

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