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PHARMACOMETABOLOMICS AND TOXICOMETABOLOMICS (C CHEN, SECTION EDITOR)

Environmental Influences in the Etiology of Colorectal Cancer: the Premise of Metabolomics

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Abstract
Purpose of Review In this review, we discuss how environmental exposures predominate the etiology of colorectal cancer (CRC). With CRC being a personalized disease influenced by genes and environment, our goal was to explore the role metabolomics can play in identifying exposures, assessing the interplay between co-exposures, and the development of personalized therapeutic interventions.
Recent Findings Approximately 10% of CRC cases can be explained by germ-line mutations, whereas the prevailing majority is caused by an initiating exposure event occurring decades prior to diagnosis. Recent research has shown that dietary metabolites are linked to both a procarcinogenic or protective environment in the colon, which is modulated by the microbiome. In addition, excessive alcohol has been shown to increase the risk of CRC and is dependent on diet, the response of microbiome, and genetic polymorphisms within the folate and alcohol metabolic pathways. Metabolomics cannot only be used to identify this modulation of host metabolism, which could affect not only the progression of the tumors but also response to targeted therapeutics.
Summary This review highlights the current understanding of the multifaceted etiology and mechanisms of CRC development but also where the field of metabolomics can contribute to a greater understanding of exposures in CRC.

Keywords Metabolomics · Colorectal cancer · Exposome · Acetaldehyde dehydrogenase · Alcohol · Microbiome

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Introduction

Cancer is a major cause of morbidity and mortality affecting all populations worldwide, with far-reaching socioeconomic consequences. Improved methods for selection of appropriate therapeutic interventions are critical to addressing this public health concern [1]. With the dawn of the millennium and through the completion of the "human genome project," concerted efforts were invested in profiling gene variants that promote tumorigenesis and multidrug resistance, in an attempt to identify biomarkers that would facilitate successful diagnostic and therapeutic interventions [2]. However, some of the challenges associated with this approach have included the genomic complexity and molecular heterogeneity of cancer, establishing the biological relevance of genetic changes to tumorigenesis, and the need for characterizing all of the associated downstream effects. Despite extensive gene characterization and the identification of thousands of mutations linked to cancer, it is increasingly apparent that only a limited number of molecular pathways that are responsible for driving carcinogenesis have been recognized, and the mechanistic basis of many genetic signatures remains largely unknown [3–5]. Indeed, for diseases that contribute to mortality in the Western population, environmental factors have an attributable risk of 80–90%; thus, both genetic and environmental

factors in tandem with gene-environment interactions need to be considered contributors to susceptibility, expression, and risk [6, 7].

Exposures play such a large role in carcinogenesis, that in 2011 the World Health Organization (WHO) calculated that 19% of all cancer deaths worldwide (more than 1.3 million people) were linked to some form of environmental stressor [8], and up to a third of those were estimated to be caused by exposures to carcinogens in the workplace [9]. However, these stark statistics are compounded by the sheer numbers of chemicals one can potentially be exposed to; in the USA, the Environmental Protection Agency Toxic Substance Control Act (EPA-TSCA) Chemical Substance Inventory contains over 85,000 different chemical moieties registered for commerce that are freely exposed to the public domain [10].

Over the past 46 years, the International Agency for Research on Cancer (IARC), acting as the world authority on determining the cancer-causing potential of different substances, has evaluated the cancer-causing potential of 900 likely candidates with approximately 100 classified as "carcinogenic to humans." The adoption of new tools that can contribute to and expedite exposure research is rapidly needed to alleviate the complexity of exposure research. Chemical profiling of blood is one such way to determine if an exposure event has taken place. Currently, just over 4500 metabolites have been identified within human blood using mass spectrometry-based metabolomics [11]. Although the vast majority documented is naturally abundant species essential to cellular function, exogenous exposure chemicals such as pesticides and environmental pollutants have been detected, quantified, and linked to a range of diseases [12, 13].

Multileveled analytical strategies that integrate genetic traits, environmental exposures, and epidemiology data can help understand complex cancer mechanisms, contributing to the generation of an expressed phenotype of exposure (Fig. 1). This concept, termed as the "exposotype," is a holistic snapshot of the multileveled downstream effects on all biological systems within the body that are influenced by an incident exposure. The process of analyzing and temporally measuring all of the exposures that an individual accrues over their lifetime is defined under the umbrella of the exposome [15], and projects such as the Human Exposome and HELIX Projects are driven by understanding the complex effects the environment plays on our personal lives [16]. Exposomics aims to analyze cumulative internal exposures (host factors which affect the cellular environment) and external exposures (toxicants and social determinants) and assess their impact on disease outcomes and risk (https://www.cdc.gov/niosh/topics/exposome/.). It is known that during the life course of an individual, a wide range of stressors can modulate every aspect of their health. These stressors come from every part of daily life and can have a profound effect on the molecular events that take place within our cells, that if left unchecked by DNA proof reading and correction mechanisms can lead to the development of unregulated cell growth and cancer (Fig. 2).

In recent years, profiling-based gene, metabolite, and protein approaches have emerged as tools capable of elucidating the interplay between epigenetic aberrations induced by environmental stimuli, and disease etiology in cancer, and thus have utility in the exposome pipeline linking exposures to biological impact [17, 18]. In addition, advancements in high-throughput analytical, statistical, and big data capabilities have paved a path for translational assessment of disease mechanisms through a systems biology approach. Consequently, to elucidate pathways and mechanisms influenced by environmental stimuli and genetic alterations, a "multileveled" approach is crucial for phenotype stratification in patient cohorts and identification of downstream molecular effectors involved in tumorigenesis. The field of metabolomics is capable of assisting by providing profiling tools that can analyze the pool of metabolites within a sample [19]. This in turn can bridge the downstream genetic and upstream environmental gap, and in combination with other profiling approaches has potential to make meaningful contributions to the field of precision medicine [20]. As an analytical technology, mass spectrometry is in a prime position to deliver on exposure analysis as it offers high sensitivity, acute selectivity, and dynamic range to analyze a broad range of metabolites. Mass spectrometry-based metabolomics, the analysis of all the low molecular weight chemical entities in a sample, can be used to directly quantify chemical exposure from human biological media such as blood, urine, and sweat, and can also investigate the downstream pathway and mechanistic consequences of an exposure event [21]. Initial work has highlighted the need for standardize analytical protocols to enable reproducible data comparisons over time and even across laboratories [22, 23]. When tested by biological questions, this approach has been applied in several situations such as workplace exposure of trichloroethylene, which has previously been linked to an increase in liver, kidney, and blood cancers [24]. By applying an untargeted metabolomics discovery approach, correlations to immunosuppression, hepatotoxicity, and nephrotoxicity inferred a deeper understanding of the molecular mechanisms of this type of exposure.

In addition, pharmacometabolomics, a tool that monitors metabolites to estimate a pharmaceutical drug response, can be used to assess inter-individual variability in tumor response and adverse events, driving the search for a means of identifying and deciphering molecular signatures that predict the behavior and drug sensitivities of distinct tumor phenotypes [25].

Herein, this review discusses the field of metabolomics and its application to understanding the etiology and mechanisms of cancer in a move towards understanding environmental exposures, gene-environment interactions, and ultimately the development of precision medicine for this disease. We focus

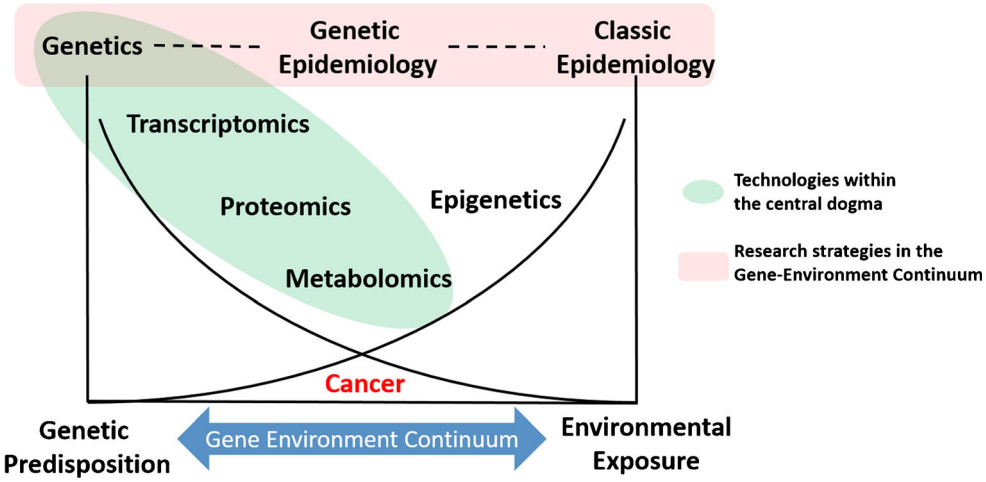

Fig. 1 Metabolomics within the gene-environmental continuum. The causation for most cancers resides at the interface of genetic and environmental exposure. By using multiple platforms, a more comprehensive analysis of the effects the exposome plays on cancer phenotype can be assessed. By analyzing the downstream metabolic products of altered gene expression, metabolomics can be used to refine mechanistic understanding of exposure while also potentially identifying novel biomarkers. Adapted from [14]

on colorectal cancer (CRC) as a prime example of complex disease in which both genetic and environmental influences (diet, alcohol, and pathogenic bacteria) are causative factors.

Metabolomics Studies on Colorectal Cancer

CRC is the third most common cancer in both males and females and the second leading cause of cancer death across both genders in the USA [26]. Only 10% of individuals that develop CRC carry germ-line mutations. These mutations result in a number of familial syndromes including Lynch syndrome, where mutations to DNA mismatch repair genes result in microsatellite instability, and familial adenomatous polyposis, which arises due to mutations in the adenomatous polyposis coli (*APC*) tumor suppressor gene [27]. Metabolic pathways linked to CRC predominantly include those involved in cellular growth. Upregulation of glycolysis and tricarboxylic cycle pathways are vital metabolic traits that are hijacked by cancer cells to enable rapid and unregulated growth; this modulation has been detected in tumor tissues from CRC patients [28, 29]. Upregulation of the pentose-phosphate pathway, another potential route for energy production, has also been detected using mass spectrometry-based metabolomics methods on HCT116 colon cancer cells [30]. Metabolic consequences of genetic aberrations in CRC have been a focus of metabolomics studies, particular models of *APC* suppression that activate β-catenin signaling [31, 32]. A comprehensive study which examined urinary metabolites and also gene expression on tumors from APC(Min/+) mice and CRC patient revealed consistent metabolic changes. The urinary metabolome revealed hypermethylated metabolites and increased polyamine synthesis and nucleic acid synthesis [33]. Other studies have used anti-proliferative agents in models of CRC to determine their effects on tumor development and metabolism. These studies examined the effect of ginseng [34, 35], rice bran [36, 37], pomegranate extract [38], natural products [39], chemotherapeutics [40, 41], and anti-microbial peptides [42] which collectively revealed decreased energy utilization and cell growth in mice and humans. However to understand the etiology of CRC, which is predominantly environmental, it is important to identify the causative agents. In addition to identification of the causative agent, the combinatorial effect of multiple exposures (internal and external) is important for risk assessment. Most studies have primarily focused on the analysis of a single exposure; therefore, it is not possible to ascertain the combined effect, let alone decipher how differences in the internal environment (inflammation, stress, genetics) respond to external environmental exposures. This is where top-down metabolomics approaches come into play, allowing for the measurement of downstream metabolites that can be related to combined exposures at a population level [43]. Metabolomics can also bridge the downstream genetic and upstream environmental gap, allowing for the analysis of both metabolites and chemical compounds that result from an exposure concurrently. This can also be directly applied to CRC patients identifying cluster of individuals that have similar profiles and outcomes.

Exposure Metabolites and Colorectal Cancer

At present, metabolomics studies that examine the effect of environmental exposures on CRC risk are few, but interest is growing due to novel insights it has provided thus far. CRC is perhaps one of the prime examples of an environmentally mediated disease; this is evidenced by studies on migrant populations that assume CRC risk of the host population within one generation [44]. Even though CRC incidence rates have

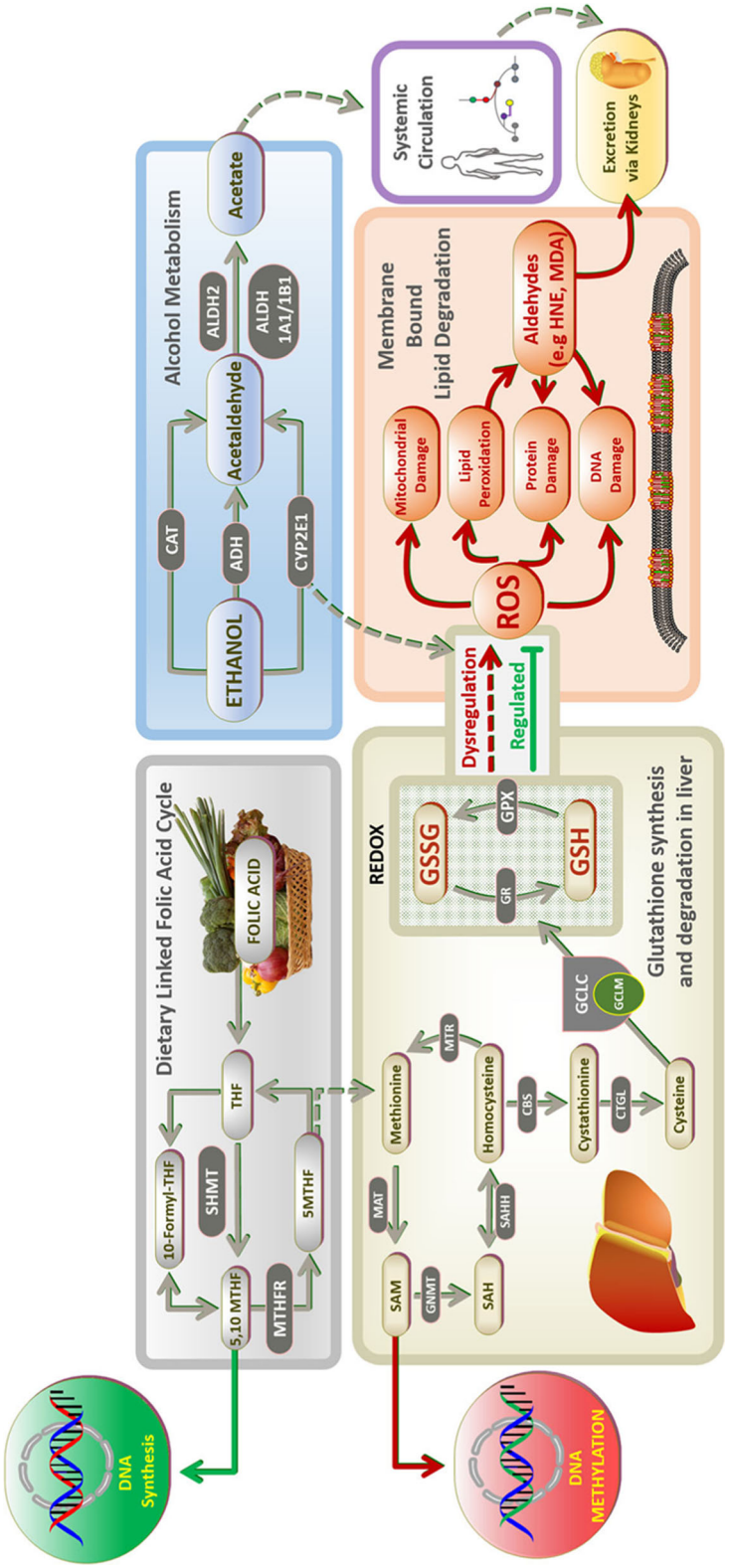

Fig. 2 The biochemical breakdown of ethanol is dependent on genetic polymorphisms in the ethanol and folate metabolic pathways. Many consecutive enzymes play important roles in maintaining redox homeostasis and protecting the cells from oxidative stress, but dysregulation within these systems can lead to the formation of reactive oxygen species (*ROS*) that can damage cellular components

been falling over the past couple of decades due to increased screening and removal of precancerous polyps, CRC incidence rates have been increasing for individuals under the age of 50, which is of high concern [45]. Thus, identification of environmental risk factors is imperative to provide intervention approaches in high-risk populations.

Specific environmental influences have been associated with CRC in epidemiological studies. They include diets rich in red, processed, and grilled meats, pre-existing diseases (obesity, inflammatory bowel diseases, type 2 diabetes), smoking, and alcohol use. Other suspected risk factors include disruption to circadian rhythms (night-shift work) and the presence and organization of pathogenic microbiota [46–48]. Dietary exposures and cancer risk is well-established, so much so that IARC recently classified one dietary constituent, processed meats and red meat, as class 1 and class 2A carcinogens, respectively [49]. As dietary risk in CRC has been discussed comprehensively in other reviews [50, 51], we will describe here the metabolites that potentially interacted with other co-exposures, leading towards increased carcinogenesis.

Ingested materials that enter the colon consist of a complex milieu of partly digested food products, xenobiotics, and pollutants. The role of the colon is to complete digestion and eliminate waste products from this milieu, a process that is aided by the host microbiome. As mentioned, processed meats (meat that has been transformed through salting, curing, fermentation, smoking) and red meats have been associated with CRC. These meats contain genotoxic N-nitroso compounds, polycyclic aromatic hydrocarbons and heterocyclic amines (upon heating). A recent study identified oxidative stress markers dityrosine and 3-dehydroxycarnitine in fecal samples from individuals that consumed red meats, supporting a causal relationship between and aberrant microenvironment in the colon that could lead to a disease [52]. Interestingly, the carcinogenic compounds found in these types of meats are also prevalent in other environmental sources such as cigarette smoke, dust, and automobile engines. This suggests that individuals exposed to high levels of these environmental pollutants could also have a higher risk of CRC. In addition to meats, high fat intake can increase the risk of CRC through increased bile acid secretion and microbial production of genotoxic secondary bile acids. Secondary bile acids have been shown to disrupt cell membranes and generate reactive oxygen species (ROS) and reactive nitrogen species causing DNA damage [53]. In addition to procarcinogenic-ingested compounds, there are a number of dietary nutrients that have been suggested to act as chemoprotectants, including the aforementioned ginseng and rice bran, which upon administration were shown to decrease cellular proliferation and carcinogenesis in vivo [34–37]. In addition to these dietary products, vitamin D has also been linked to decreased CRC risk [54]. Vitamin D is a ligand for the nuclear vitamin D receptor (VDR), which upon activation causes downstream regulation of a multitude of genes involved in cellular proliferation and tumor angiogenesis. One of these genes (CYP3A4) is of particular importance as it is involved in the elimination of secondary bile acids; thus, downregulation of VDR could increase the risk of CRC in individuals with a high fat diet [55, 56]. Metabolomics studies on mouse models of *Vdr* inactivation showed patterns of increased primary and secondary bile acid production associated with inflammatory bowel diseases and CRC [57]; however, metabolomics studies have not been carried out on CRC patients to determine the association. Fiber is also a well-established chemoprotectant involved in elimination of secondary bile acids and increasing the passage and weight of stools. Moreover, one of its most important properties is through the actions of its metabolic products, butyrate, propionate, and acetate, which are produced by microbial fermentation in the colon. Butyrate in particular has been shown to play a major role as an anti-proliferative agent, also increasing apoptosis and the production of healthy bacteria. Metabolomics studies carried out on feces collected from patients with CRC have consistently shown decreased butyrate levels compared with controls [58–60]. Folate, derived from fruits and vegetables, is an essential intermediate in one-carbon metabolism and increases the production of *S*-adenosylmethionine (SAM). SAM is a ubiquitous methyl donor, enabling the metabolism of polyamines, DNA synthesis, and DNA methylation. Individuals with folate deficiency have increased DNA hypomethylation and impaired DNA synthesis; in addition, low levels of folate accelerate alcohol-induced production of oxidative stress as described below. Given the evidence for the roles of dietary metabolites in CRC, further analysis is required to assess the biological effects of these metabolites.

Moreover, it is clear that co-exposure of diet with other environmental factors augments or ameliorates their effects. One such intricate balance has emerged between the diet, alcohol, and microbiome.

Alcohol and Colorectal Cancer

Excessive alcohol consumption and its associated health and societal problems vary worldwide; however, the burden of disease and death is a significant public health problem in nearly all countries [61]. There is evidence from epidemiological studies to show that alcohol consumption is associated with CRC [62]. A 2011 meta-analysis showed a higher association with CRC among subjects with heavy drinking ($RR_{\geq 4\ drinks/day} = 1.52$ (95% CI, 1.27–1.81)) compared with moderate drinkers ($RR_{2\ to\ 3\ drinks/day} = 1.21$ (95% CI, 1.13–1.28)). In addition, this link was found to be stronger in individuals of East Asian decent (heavy drinkers, RR = 1.81 (95% CI, 1.33–2.46); $p = 0.04$) [63]. The same conclusion was

drawn from a 2006 meta-analysis study, which included more than 6300 patients; heavy drinkers had a higher risk of CRC than moderate drinkers [64]. Age, gender, volume and quality of alcohol consumed, and pattern of drinking have all been shown to modulate risk [61, 62].

Genetic polymorphisms for enzymes in the alcohol and folate metabolic pathways play a major role in CRC risk. Ethanol is metabolized to acetaldehyde via alcohol dehydrogenases (ADH), catalase (CAT), and CYP2E1 enzymes. It is further oxidized via acetaldehyde dehydrogenase (ALDH), isoforms 1A1, 1B1, and 2 to acetate and eliminated by the liver [65, 66]. Ethanol-induced cancer is closely linked to the pathways involved for its metabolism and is mainly attributed to the production of acetaldehyde which has been classified as a carcinogen by IARC [67]. Evidence for the association between ethanol, acetaldehyde, and cancer is derived from epidemiological studies, as well as from mechanistic data from the rodent models. Ethanol oxidation by ADH and CYP2E1 results in the production of acetaldehyde; however, the latter induces the generation of ROS (Fig. 2) [68]. Compared with wild-type mice, the development of fatty liver and oxidative stress is alleviated in Cyp2e1−/− mice [69, 70]. Moreover, there is evidence to support a genotoxic mechanism whereby acetaldehyde reacts with DNA bases, forming DNA adducts, resulting in genetic mutations. N^2-ethyl-2′-deoxyguanosine (N^2-ethyl-dG), the major stable acetaldehyde DNA adduct, has been reported at high levels in alcoholics. DNA adducts can also be produced by ROS; 4-hydroxynonenal (4HNE) is a lipid peroxidation product that reacts with deoxyadenosine or deoxycytidine to form stable exocyclic etheno-DNA adducts (Fig. 2) [71–74]. However, large inter-individual variability exists in the presence of etheno-DNA adduct in alcohol consumers, and significant correlations have not been established [75].

There is also evidence that polymorphisms in methylenetetrahydrofolate reductase (MTHFR) contribute to a lower risk of CRC. The CT/TT genotype shifts folate metabolism away from DNA methylation to DNA synthesis. Moderate to heavy alcohol drinkers with this genotype have a lower risk of CRC when compared to never/occasional drinkers with the CC genotype (OR = 0.68 (95% CI, 0.47–0.98)) [76]. Furthermore, MTHFR plays an important role in the one-carbon metabolic pathway converting 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate (Fig. 2) and directs folate pools towards methionine in the transsulfuration pathway. Methionine is the precursor of the sulfur-containing amino acid cysteine (cystathionine β-synthase (CBS) catalyzes homocysteine conversion to cysteine). This pathway leads into the synthesis of glutathione (GSH) via a heterodimer enzyme comprising a catalytic glutamate-cysteine ligase (GCLC) and a modifier (GCLM) subunit (Fig. 2). GSH plays a key role in maintaining redox homeostasis and protecting the cells from oxidative stress. A recent study utilizing global knockout *Gclm* mice found that the cells were protected against hepatic steatosis, despite showing increased oxidative stress, suggesting that an adaptive response was triggered through activation of the AMPK pathway [77]. The abovementioned metabolic pathways have been the subject of multiple studies on cancer metabolism; however, metabolomics studies are required to fully understand the effect of alcohol on the folate pathway and the role of MTHFR [78].

Acetaldehyde concentrations are generally quite low in tissues, however, levels can increase under a combination of large volumes of ethanol ingestion and inefficient ethanol-metabolizing enzymes; in the colon, alcohol levels can reach the same as those seen in blood [75]. ALDH2 is crucial for acetaldehyde catabolism, since most of the acetaldehyde generated is eliminated by actions of this enzyme. The mutant *ALDH2*2* allele encodes a catalytically inactive subunit, whereas *ALDH2*1/2*2* encodes only 6.25% of the active enzyme. Individuals carrying the mutant allele are slow acetaldehyde metabolizers and subsequently accumulate large pools of the metabolite [79]. Studies on *Aldh2*−/− mice found that ethanol treatment increased plasma acetaldehyde levels compared with the wild type [80, 81]. Approximately 40% of individuals of East Asian descent carry the inactive variant *ALDH2*2*, resulting in extremely low ALDH2 activity. Individuals with the *ALDH2*2* allele have increased risk for developing cancer of the upper aerodigestive tract, as well as oropharyngeal, laryngeal, and esophageal cancers [82, 83]. Another recent study found that 39 out of 40 human samples with colonic adenocarcinoma were positive for ALDH1B. Since the expression of this protein is very low in healthy colon, the dramatic increase observed in the tissues demonstrates a link between elevated ALDH1B1 and CRC [86]. Increased plasma acetaldehyde levels have also been measured in Aldh1b1−/− mice after a single dose of ethanol [65]. A strong association has also been seen in individuals that have polymorphisms in ADH1B and CRC [62, 84]; *ADH1B*1/*1* carriers (n = 246) were shown to have a much slower metabolism of ethanol compared with *ADH1B*2* carriers (n = 559) [85].

In order to evaluate the association between alcohol consumption and CRC in human populations, studies currently rely on self-reported questionnaire data, which is subject to misclassification due societal pressure. Metabolomics could be used to strengthen the causal association through the use of non-invasive biomarkers which could be used as an approach for risk assessment. A recent study analyzed serum from two population cohorts (case-control) to quantify previously identified biomarkers of alcohol consumption [87]. Unfortunately, no association was seen between these biomarkers and CRC. This may have been a consequence of the time frame from alcohol consumption to sampling, and a lack of personal history on the nature of the individual's lifetime drinking, both of which were not known. However, this study highlights the potential and considerations for metabolomics analysis for assessing alcohol consumption in relation to CRC, particularly to assess the validity of

questionnaire data. Other metabolomics studies have identified biomarkers of alcohol consumption, which are promising and need validation in additional cohorts [88]. In addition to biomarkers, mechanisms of ethanol-induced injury that lead to CRC can be investigated through further investigation of metabolic pathways, revealing interactions between alcohol and other co-exposures to understand inter-individual variability.

Microbial Co-exposure and Mechanisms of Colorectal Cancer

One important consideration for almost all exposures is the interaction with the microbiome. For the past decade, the microbiome has been under surveillance as a potential mediator of CRC. It is known that the colonic microbiome is responsible for co-metabolism of dietary and environmental compounds that enter the colon, and the resultant metabolites produced can have either carcinogenic or tumor-suppressive properties as well as regulating microbial growth and diversity. One example is microbial fermentation of dietary fiber which produces short chain fatty acids as mentioned previously. These acids could effectively lower the pH of the colon if produced in excess; however, bicarbonate secretion, controlled by cellular carbonic anhydrase, ensures that colonic pH is maintained at approximately pH 7.4 [89]. This balance can be affected by multiple processes, including bacteria exotoxins which can stimulate bicarbonate secretion [90], or by disease, cancer cells produce elevated levels of lactic acid and protons which modify carbonic anhydrases enabling the cancer cells to survive [19, 91]. In addition to host cellular processes, environmental changes caused by switches in diet, microbial infection, or drug treatment can cause a modulation of the microbial communities within the colon. Dietary changes can rapidly alter the growth of different bacterial species, changing disease risk and metabolite production. This was exemplified in an investigation of the microbiome and metabolome in subjects given a 2-week dietary exchange [92]. A switch from a high fat, high protein, and low fiber diet, to the inverse, resulted in increased saccharolytic fermentation and butyrogenesis with decreased secondary bile acid production. In addition, a decrease in proliferative and inflammatory biomarkers was observed in the colonic mucosa, revealing that dietary exposure can alter the microbiome, metabolites, and disease risk [93].

Previous research has shown that several invasive pathogenic bacteria are linked to CRC, most notably enterotoxigenic *Bacteroides fragilis* [94], *Fusobacterium nucleatum* [95, 96], and *Escherichia coli* strain NC101 [97]. Mechanisms of action reveal DNA damage, inflammation, and immune-cell infiltration in models; however, direct causation has yet to be ascertained in CRC patients [98]. It is also important to consider how the community of bacteria interacts as a whole; this is evident from studies that have identified chronic bacterial biofilms in CRC. Chronic biofilms are conglomerates of bacteria encased in a polymeric matrix, which colonize and breech the mucus barrier. Biofilms in CRC cause a state of increased cellular proliferation and inflammation but do not contain higher abundances of pathogenic strains compared with tumor-associated bacteria which are not within a biofilm [47]. Interestingly, normal colon tissues that have biofilms have similar bacterial diversity associated with CRC tissues than with normal colon tissues without biofilms, indicating a stepwise progression from a dysbiotic microbiome to biofilm production and carcinogenesis [99]. Furthermore, metabolomics was used to examine the metabolism of biofilms in CRC and revealed biofilm-associated metabolites [46], indicating that biofilms produce metabolites that could affect the tumor microenvironment and response to therapeutics [100]. However, what is not clear is the exposure that triggered biofilm formation and tumor development in this study. A new paradigm developed for exposomic approaches suggests a meet-in-the-middle approach to tackle this problem [101]. Prospective cohort studies that measure exposures and preclinical response can be used to identify associated biomarkers. These exposure biomarkers can then be used to mine retrospective case-control studies to essentially identify casual relationships [102].

As aforementioned, alcohol can result in oxidative stress within the colon and a progression to CRC. The microbiome is also involved in the metabolism of alcohol; microbial co-oxidation of ethanol increases the production of acetaldehyde. Microbial genera involved in ethanol oxidation have been identified as gram-positive *Ruminococcus*, *Collinsella*, *Coriobacterium*, and *Bifidobacterium* and gram-negative *Prevotella* [103]. *E. coli* has also been linked to the accumulation of acetaldehyde in the intestinal lumen [104]. Due to the ensuing oxidative stress caused by chronic alcohol exposure, obligate anaerobes are less likely to prevail than other species that are more tolerant to the microenvironment, such as *Proteobaceria*, a species linked to inflammation [105]. For the host, this inflammation is likely to be exacerbated by downstream acetaldehyde production. Endotoxin circulation from gram-negative bacteria subsequently leads to increased intestinal barrier permeability, a facet compounded by bacterial overgrowth observed in moderate drinkers [106]. The creation of such dysbiotic environments has also been shown to be vital factors in the promotion of other alimentary cancers [107] and even liver disease [108]. Interestingly, modulation of bacterial species in the gut has even been shown to play a psychological role in which the gut-brain axis has been altered by microbiotic communication affecting gut-barrier function and influence behavior in alcohol dependence [109].

Therapeutics and Pharmacometabolomics in Colorectal Cancer

Since the evolution of metabolomics in 1998 [110], the development and growth of the sub-discipline of pharmacometabolomics [111] has blossomed to such an extent that a community-led White Paper has called for US Government inclusion of the area in to the highest level of funding considerations [112]. This confidence has been driven by the potential to deliver in areas that genome-based research alone has failed to achieve a full understanding of and has heralded the post-genomic diagnostic era in which detailed knowledge of expressed phenotype is vital. Applications include revealing primary and secondary markers of disease states, providing in-depth understanding of underlying molecular mechanisms of diseases, and the potential to uncover biomarkers activated by drug treatment thus identifying response phenotypes [112].

One area of use for these strategies is cancer, and several reviews have been written that provide comprehensive background on the area [113–117]. As the lifetime risk of developing CRC in the USA is 1 in 21 (4.7%) for men and 1 in 23 (4.4%) for women [118], it is no surprise that concerted efforts have been made to apply these methodologies to this disease. Identification of early onset biomarkers and prognostication has been a focus of CRC researchers since early detection and diagnosis can make curing the disease easier. One set of metabolites, the acetylated polyamines, monoacetylspermidine and N^1,N^{12}-diacetylspermine, are constantly upregulated in biological fluids and tissues of CRC patients [46, 119–124]. These metabolites are end products of polyamine metabolism transported from the cell for excretion. Polyamines have been associated with the development of CRC, and ornithine decarboxylase (ODC), the first enzyme in the polyamine synthesis pathway, is a target for the chemotherapeutic difluoromethylornithine (DFMO). DFMO inactivates ODC and suppresses tumor formation [125]. Therefore, this metabolic pathway is of particular interest for drug development for CRC patients, and DFMO is currently under assessment in a number of clinical trials. Serum amino acids [126] and urinary nucleosides [127] have also been identified as candidates for future assay design alongside methods for modeling stratified markers for disease staging [128]. The ultimate aim for all these approaches is understanding mode of action of disease better and eventually creation of a rapid and robust blood or urine-based screening test that can be routinely used within the clinic and thus reduce the number of patients with a poorer prognosis.

Upon treatment with chemotherapeutics, inter-patient variability in response can be large and lead to higher levels of toxicity to some patient sub-groups alongside lower efficacy of the active drug. Pharmacometabolomics profiling techniques have already shown that drug toxicity can be predicted in CRC patients treated with the chemotherapeutic capecitabine [129] in which a panel of low-density lipoprotein-derived lipids predicted a higher toxic response over the treatment period. The efficacy of such drug treatments have also been shown to be markedly affected by a host's microbiome with researchers also calling for measurement of microbiome activity to be included as an integral part of pharmaceutical development [130]. Ultimately, the greatest impact of any metabolomics technology in CRC investigation lies within the integration of metabolic data into multidimensional datasets forming a systems wide approach. Only by combining information from pharmacogenomics [131], pharmacoproteomics [132], and transcriptomics experiments alongside drug pharmacology-based pharmacokinetic and pharmacodynamics processes [133] can the fullest picture of how an individual will respond to drug intervention be painted.

Conclusion

Metabolomics is a vital tool for evaluating exposures and their impact on biological outcomes, particularly in a disease with a multifaceted etiology such as CRC. Using metabolomics within the context of the exposome could identify subsets of individuals that have similar exposure history and related internal biological underpinnings, thus providing entry points for precision medicine approaches. Pharmacometabolomics, a discipline that monitors metabolites to understand the response of a pharmaceutical drug leads itself to exposomics and precision medicine [134]. However, caution exists in that the complex makeup of the internal environment must be known before administration of the drug, as has been evidenced by interactions of the microbiome with immunotherapeutics, dramatically affecting their efficacy [135]. Thus, drugs must be tailored to respond to the genetic underpinnings of the disease manifestation as well as from onslaughts from the internal environment. This will dramatically improve drug efficacy and move towards precision medicine approaches. Therefore, oncology metabolomics can be applied in diagnostic techniques, studies of disease pathophysiology, prognostication, and prediction and assessment of response to therapy [136]. While profiling-based technologies at all stages of the central dogma are currently specialized and complex in nature, signatures identified by these approaches can be incorporated into miniaturized "lab-on-chip" systems applicable to the clinic in the future [137]. Thus, metabolomics can be applied to studies on CRC to uncover mechanisms of cancer development and identify gene-environment interactions. Moreover, metabolomics can aid in assessment of disease severity and response to pharmacological interventions in the clinic via pharmacometabolomics and even be used to identify patients in early prediagnostic stages of the disease. Ultimately, this work is leading to improved awareness of environmental risk factors, early diagnosis, and outcomes for CRC patients.

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Compliance with Ethical Standards

Conflict of Interest There are no conflicts of interests within this article.

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