Review Article

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Research progression of blood and fecal metabolites in colorectal cancer

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Abstract

The development of colorectal cancer (CRC) is correlated with metabolic changes, suggesting great potential of metabolites to be diagnostic and prognostic biomarkers for clinical management. A large variety of metabolites have recently been identified due to their oncogenic role and clinical significance. Although the results may vary within studies due to their diversity and complexity, these biomarkers profoundly reflecting intestinal homeostasis and disease status can be further developed as noninvasive diagnostic tools in complementary to traditional approaches such as colonoscopy, particularly for early population-based screening. This review focuses on the potential clinical utilities of metabolites as novel biomarkers in CRC, and discusses the metabolites-directed strategy for early diagnosis.

Keywords: Fecal metabolites, Blood metabolites, Colorectal cancer, Study progress

Colorectal cancer (CRC) is the third most common cancer in both men and women. In China, its incidence has reached ~37.63 per 100,000 in 2015 according to the latest survey[1]. CRC is initiated by both internal (genetic inheritance) and external factors (risk factors include lifestyle and the environment). Genetic inheritance is a major cause of CRC in patients with familial adenomatous polyposis and increased understanding of the adenoma-to-carcinoma progression may help us find new early diagnostic methods for these patients[2]. External factors include smoking, drinking, high-fat and low-fiber diets, intestinal microbiota dysbiosis, or alcohol. General obesity and lack of physical activity could also increase the incidence of CRC[3–8]. For example, tobacco smoke contains more than 40 carcinogens, including benzopyrene, nitrosamines, polycyclic aromatic hydrocarbons, nitroso toluene and nickel. Studies have found that nicotine can promote colon cancer in vitro and in vivo and the main mechanism is that the phosphorylation of epidermal growth factor receptors and c-Src which increase the expression of 5-lipoxygenase[9]. Smoking also activates various oncogenesis signalling pathways in sporadic CRC[10]. Moreover, the effect of smoking on intestinal microbiota also contributes cancer progression[11]. Current studies have found that alcohol increases CRC risk through 2 metabolic pathways: (1) ethanol is oxidized to acetaldehyde to induce malignant transformation[12]; (2) alcohol serves as a folic acid antagonist that induces folic acid deficiency and subsequently results in DNA hypomethylation and abnormal DNA synthesis[13,14]. Conversely, high folate intake or low alcohol consumption may reduce the risk of CRC[15]. Diet can regulate the composition of intestinal microbes. For example, long-term high-protein high-fat diets can increase the abundance of bile-tolerant microorganisms like Bacteroidetes and reduce the butyrate-producing bacteria *Firmicutes*[16], while carbohydrate diets can increase the abundance of *Prevotella*[17]. Diet-dependent microbial colonization can also alter host epigenetics: a "Western-type" diet inhibits the effects of polysaccharide dietary-related microorganisms on host chromatin, and the production of short-chain fatty acids (SCFA) in microorganisms, while sterile mice supplemented with SCFA can be reobserved with epigenetic phenotypes associated with colonization[18]. A study has shown that low dietary fiber intake can reduce the risk of CRC by 40%[19]. Recently, a meta-analysis of 25 prospective studies has shown that as dietary fiber intake increased by 10 g/d, CRC risk decreased by 10% [95% confidence interval (CI), 6%–14%][20]. The intake of processed red meat can promote the development of CRC through the metabolism of mutagens (such as heme iron, heterocyclic amines and N-nitroso compounds), and induce carcinogenesis by lipid peroxidation[21]. In addition, high red meat intake promotes the growth of sulfate-reducing bacteria that produce hydrogen sulfide, which is a genotoxic agent[22]. It was also found that there was a positive correlation between the consumption of red meat and the bacterium (*Bacteroides*, *Alistipes*, *Escherichia*, *Parvimonas*, *Bilophila*, and *Fusobacterium*), which were enriched in carcinoma samples, compared with both healthy and advanced adenoma samples[23]. This may be related to the increased abundance of amino acid consumed in bacteria induced by long-term red meat diet[23]. Therefore, it can be concluded that CRC development is a multifactorial complicated process and identification of potential driving factors may provide great benefits to CRC prevention and treatment.

The 5-year survival rate is 93% for CRC patients within stage I, while it drops to 8% for those within stage IV, strongly supporting the necessity of early diagnosis and risk population screening[24]. Currently, the US Preventive Services Working Group recommends fecal occult blood tests (FOBTs), flexible

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sigmoidoscopy, or colonoscopy screening for early detection of CRC[25]. However, numerous limitations have been found in these detecting methods. Furthermore, although colonoscopy is widely accepted as the gold standard for precancerous or CRC detection, this invasive approach often frequently causes some side-effects such as abdominal pain and is inconvenient for general population screening. Recent studies based on blood or fecal samples have held promise for noninvasive biomarkers in CRC detection such as microRNA and methylated SPET9, but few of them have been actually applied in clinical management largely due to their inferior sensitivity or insufficient validations[26]. Therefore, searching for other potential biomarkers that can improve the current diagnostic system is of great urgency and practical meaning.

Metabolic changes are also an important process of tumor development and many metabolites are found to play an important role in tumor cell proliferation, apoptosis, and invasion. However, in CRC, although recent work have proposed some metabolites as clinical biomarkers, our knowledge of this field is currently limited and related clinical validations are lacking. Hence, a further comprehensive understanding of these metabolites in CRC field will not only be essential for systematic oncogenic researches regarding metabolism, but also help clinicians establish a novel metabolite-based noninvasive diagnostic strategy for CRC patients.

Metabolic changes in CRC

The complexity of the biological mechanism of CRC has retarded the advance in diagnosis and treatment. Much effort has been made to explain the pathogenesis of CRC: genetic inheritance, molecular signalling pathways and metabolic correlations, among which metabolomics field is poorly investigated. Metabolomics, an approach that involves the comprehensive profiling of the full complement of endogenous metabolites in a biological system, has demonstrated its great potential for early diagnosis and personalized treatment of various cancers including CRC[27]. Small molecules, such as metabolic substrates and products, lipids, small peptides, vitamins and other protein cofactors, interact with each other and with other biological macromolecules, forming a comprehensive biochemical network, known as the metabolome[28]. Metabolites are the final product of cell metabolism, and their component changes could reflect the abnormal status of tissues. Therefore, a better understanding of these changes may improve diagnosis and discover new targets for the treatment and prevention of CRC. Interestingly, accumulating evidence suggests that intestinal microbiota contributes to the onset of CRC, which is not only via the pro-carcinogenic activities of specific pathogens, but also due to the influence of bacterial metabolites[29]. The identification of cancer-associated bacteria and understanding the contributions of microbial metabolism in health and diseases remains challenging. However, such studies are likely to greatly advance the diagnosis[30]. Thus, we summarized these studies on fecal, blood metabolites and intestinal bacterial metabolites in CRC patients in **Table 1**. Two investigators (C.K. and R.G.) independently performed computerized literature searches of the PUBMED and WEB OF SCIENCE databases to identify relevant studies published after 2009. The following text and/or medical subject heading terms were used: (1) "metabolite" OR "metabolomics" (2) "serum" OR "plasma" OR "blood" OR "fecal" and (3) "colorectal" OR "colon" OR "rectal" OR "large bowel". The search strategy generated 2670 citations, of which 27 were considered of potential value; the full texts of these publications were retrieved for detailed evaluation (**Fig. 1**).

Changes in fecal metabolites

Given its contact with and transient stay in the colon and rectum, stool carries a lot of valuable information that reflect the health/disease status of the colon and rectum. In addition, the extraction of fecal metabolites is an inexpensive, reproducible and effective detecting method that enables identification of potential biomarkers indicating CRC occurrence and progression.

Certain dietary nutrients, such as phosphatidylcholine, choline and carnitine, are processed specifically by the gut microbiota to produce trimethylamine. Trimethylamine is absorbed in the gut and converted to trimethylamine oxide (TMAO) in the liver by hepatic flavin-containing monooxygenases[58]. In humans, foods such as meat and eggs have been associated with increased levels of TMAO, which in turn lead to increased risk of major cardiovascular events in patients with proven coronary heart disease. These findings further support the involvement of intestinal microbiota in TMAO production[58]. Xu and colleagues investigated the interaction between dietary and microbial metabolism, as well as the epigenetics of the disease, and found that TMAO-related genes and CRC-related genes share a common pathway in the immune system, cell cycle, cancer pathways and Wnt signaling pathways. Thus, TMAO may be an important intermediate biomarker that links dietary meat, fat and gut microbiota metabolism to the risk of CRC[59].

The production of insoluble fiber is known as SCFA, which show essential roles in the homeostasis of intestinal microenvironment and other physiological activities[60]. SCFA are mainly indigested polysaccharide and bacterial fermentation products, and only a small part is from the metabolic products of proteins and amino acids. SCFA and, in particular, butyrate are the main sources of energy of colonic epithelial cells[61]. Butyrate may have direct effects on gene expression as a histone deacetylase inhibitor, and has been shown to affect DNA methylation and the proliferation/differentiation of colonic epithelial cells. Other studies have demonstrated the effect of butyrate on resistance to cancer, anti-inflammatory and anti-oxidation. The reduction of butyrate in fecal is usually due to the decrease in butyrate production by intestinal microbiota[62]. In fact, according to host genetics and intestinal microbiology, SCFA may even serve as an anticancer drug, due to its effect on promoting cell apoptosis and cell differentiation[63]. Singh et al[64] found that butyrate and nicotinic acid are metabolites of intestinal microflora, and both mediate the protective effects of intestinal inflammation and CRC via the GPR109a signalling pathway. Amiot and colleagues investigated the distinction of fecal samples between CRC and healthy controls using proton nuclear magnetic resonance (^{1}H-NMR) to identify biomarker candidates. They also found that SCFA including valerate, acetate, propionate and butyrate were increased in CRC groups, and the essential substrates for tricarboxylic acid cycling such as β-glucose, glutamine and glutamate were decreased in CRC patients[54]. In addition, Chen et al[65] analyzed the fecal microbiota community by 454 pyrosequencing based on 16S ribosomal RNA, and found that lower dietary fiber patterns and

Table 1

Changes in blood and fecal metabolites in CRC patients.

References	Sample	Tools	CRC	Control	Increased in CRC	Decreased in CRC
Nishiumi et al[31]	Plasma	GC-QqQMS	282	291	Pyruvic acid-meto-TMS, glycolic acid-2TMS, fumaric acid-2TMS (/SI), ornithine-4TMS (/SI)	Palmitoleic acid-TMS, tryptophan-3TMS (/SI), lysine-4TMS, 3-hydroxyisovaleric acid-2TMS
Uchiyama et al[32]	Serum	CE-TOFMS	56	60	3-hydroxybutyric acid	Isovaleric acid, ornithine, benzoic acid, and amino acids His, Lys, Trp
Kuhn et al[33]	Plasma	FIA-MS/MS, LC-MS/MS	163	774	Phosphatidylcholines	Glycine, Ser, lysophosphatidylcholines
Farshidfar et al[34]	Serum	GC-MS	320	254	Ethylene glycol, lactic acid, stearic acid, 3-hydroxybutyric acid, 2-hydroxybutanoic acid, phenylalanine, isoleucine, citric acid, lysine, glycine	Threonic acid, cystine, arabinose, malonic acid, nonane, glycerol, D-ribose 5-phosphate
Zhu et al[35]	Serum	LC-MS/MS	20	—	Glucose 1,6-bisphosphate (G16BP), fructose 1,6-bisphosphate (F16BP), 1-methylguanosine, 2-aminoadipate, citraconic acid, N2, N2-dimethylguanosine oxaloacetate; cis-aconitate; succinate; homogentisate; methylmalonate; adenine; urate	Galactose, pyruvate, cystathionine, 3 nitro-tyrosine, ornithine, methyl succinate
Chen et al[36]	Serum	LC-TOFMS	20	20	Diacylglycerols	Lysophosphatidylcholines, phosphatidylcholines (PCs)
Zhu et al[37]	Serum	LC-MS/MS	66	92	Glycocholate, hippuric acid, glycochenodeoxycholate, leucic acid, maleic acid, hydroxyproline/aminolevulinate, glyceraldehyde	Histidine, malonic acid/3-hydroxybutyrate (3HBA), methionine, linolenic acid, 2-aminoadipate, N-acetylglycine
Zamani et al[38]	Serum	^{1}H-NMR	33	33	Glycine	Pyridoxine, orotidine, S-adenosylhomocysteine, pyridoxamine, glycocholic acid, beta-leucine, 5-methylcytidine, taurocholic acid, 3-hydroxybutyric acid, 7-ketocholesterol
Cross et al[39]	Serum	LC-MS, GC-MS	254	254	Glycochenodeoxycholate (women only)	Leucyl-leucine, fumarate
Bae et al[40]	Plasma	LC-MS/MS	835	835	TMAO, choline, homocysteine, dimethylglycine	Betaine, folate, PLP, fitamin B_{12}
Lee et al[41]	Serum	MS with low mass ions	30	30	Phosphoenolpyruvate	—
Tan et al[42]	Serum	GC-TOFMS, UPLC-QTOFMS	101	102	2-oxobutanoic acid, β-hydroxybutyrate, tetrahydrogestrinone, glycerol	5-hydroxytryptamine, tryptophan, aspartate, LysoPC, fumarate, 4-hydroxystyrene, phenylalanine
Li et al[43]	Serum	DI-ESI(±)-FTICR-MS	52	52	Lysophosphatidylcholine, phosphatidylcholine, lysophosphatidic acid	Palmitic amide, oleamide, hexadecanedioic acid, octadecanoic acid, eicosatrienoic acid, myristic acid
Nishiumi et al[44]	Serum	GC-MS	60	60	Lactitol, taurine, mMeso-erythritol, gulcono-1,4-lactone, dopamine, 3-hydroxybutyrate, fructose, oxalate, kynurenine, 2-hydroxybutyrate, glutamic acid	Acetylsalicylic acid, nonanoic acid, ribulose, lactic acid, ribose
Leichtle et al[45]	Serum	FIA-MS/MS	59	58	—	Lysine, alanine, aspartic acid, glycine, histidine, leucine/isoleucine, methionine, sarcosine, threonine, tyrosine, valine
Bertini et al[46]	Serum	^{1}H-NMR	153	139	3-hydroxybutyrate, acetate, formate, glycerol, lipid (-CH2-OCOR), N-acetyl signal of glycoproteins, phenylalanine, proline	Alanine, citrate, creatine, glutamine, peptide NHs, lactate, leucine, pyruvate, tyrosine, valine
Ikeda et al[47]	Serum	GC-MS	12	12	L-glutamine, L-alanine, glucuronoic lactone	—
Miyagi et al[48]	Plasma	HPLC-ESI-MS	199	995	Gln, Pro, Gly, Ile	Trp, His, Thr, Ser, Cit, Val, Met, Tyr, Orn, Arg
Ritchie et al[49]	Serum	QQQ-MS/MS	248	1073	—	28 carbon-containing hydroxylated polyunsaturated ultra long-chain fatty acids
Ritchie et al[50]	Serum	FTICR-MS, LC-MS/MS, NMR	222	220	—	28-36 carbon-containing hydroxylated polyunsaturated ultra long-chain fatty acids
Ma et al[51]	Serum	GC-MS	30	—	L-tyrosine	L-valine, 5-oxo-L-proline, 1-deoxyglucose, D-turanose, maltose, arachidonic acid, hexadecanoic acid
Qiu et al[52]	Serum	GC-TOFMS, UPLC-QTOFMS	64	65	Pyruvate, lactate	Lysine, leucine, threonine, valine, tyrosine, uridine
Lin et al[53]	Fecal	^{1}H-NMR	68	32	Succinate, proline, alanine, dimethylglycine, valine, glutamic acid, leucine, isoleucine, lactic acid	Acetate, butyrate, propionate, glucose, glutamine
Amiot et al[54]	Fecal	^{1}H-NMR	33	22	Valerate, acetate, propionate, and butyrate	β-glucose, glutamine, and glutamate
Goedert et al[55]	Fecal	HPLC-GC/MS-MS	48	102	Palmitoyl-sphingomyelin, mandelate, P-hydroxy-benzaldehyde	Acetaminophen, tocopherols, sitostanol, 3-dehydrocarnitine, pterin, N-2-furoyl-glycine, P-aminobenzoate
Phua et al[56]	Fecal	GC/TOFMS	11	10	—	Nicotinic acid, fructose, linoleic acid
Monleon et al[57]	Fecal	NMR	21	11	Leucine, proline, cysteine	Acetate, butyrate

CRC indicates colorectal cancer; CE, capillary electrophoresis; FTICR, Fourier transform ion cyclotron resonance; GC, gas chromatography; LC, liquid chromatography; MS, mass spectrometry; NMR, nuclear magnetic resonance; TMAO, trimethylamine oxide.

consistently lower SCFA production were observed in the advanced colorectal adenoma (A-CRA) group. Moreover, butyrate and butyrate-producing bacteria were more prevalent in the subgroup of healthy control subjects with high fiber intake, compared with subjects in the low-fiber healthy control subgroup and high-fiber A-CRA subgroup[65]. In addition, SCFA has also been found to affect the function of immune $CD4^+$ T cells, which play an important role in chronic intestinal inflammation and tumorigenesis. For example, Singh et al[64] found that GPR109A, a receptor for butyrate, mediates the promotion of anti-

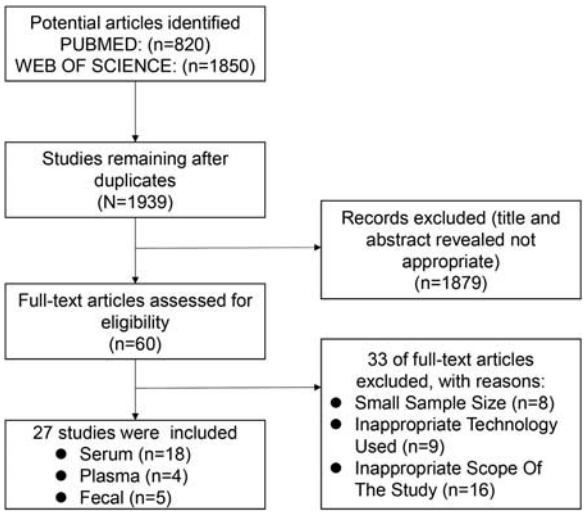

Figure 1. Flow chart of the study selection.

inflammatory properties in colon macrophages and dendritic cells, which enable them to induce differentiation of Treg cells and IL-10-producing $CD4^+$ T cells. In addition, butyrate and acetate can increase intracellular IL-18 levels in a time and dose-dependent manner, suggesting that these intestine components may have a potential regulatory role on T-cell–mediated inflammatory and intestinal tumor formation conditions[64].

Colorectal adenoma is generally considered to be a classic sporadic CRC precancerous lesion, and the abundance of intestinal flora and change in its metabolite level in colorectal adenoma are also important for predicting the occurrence and development of CRC. Hale et al[66] analyzed 16S rRNA gene sequences from the fecal microbiota of patients with and without adenomas, and found that multiple taxa were significantly more abundant in patients with adenomas, including *Bilophila*, *Desulfovibrio*, proinflammatory bacteria in the genus *Mogibacterium*, and multiple *Bacteroidetes* species; which are predicted to increase the production of primary and secondary bile acid, as well as starch, sucrose, lipid, and phenylpropanoid metabolism. These data imply that increased sugar, protein and lipid metabolism, along with increased bile acid production, may promote a colonic environment that supports the growth of bile-tolerant microbes such as *Bilophilia* and *Desulfovibrio*. In turn, these microbes may produce genotoxic or inflammatory metabolites such as H2S and secondary bile acids, which could play a role in catalyzing adenoma development, and eventually CRC[66].

According to the synthesis of the analysis results of CRC fecal metabolites in different studies, it was found that certain fecal metabolites, which mostly belong to amino acids, inorganic acids, aldehydes, fatty acids, fatty acid esters and polyols, are involved in the disruption of normal bacterial ecology, the malabsorption of nutrients, increased glycolysis and glutaminolysis[53]. Compared with healthy controls, an increase in glutamate, proline, cysteine, sarcosine, leucine, isoleucine, valine, succinate, p-hydroxybenzaldehyde, palmitoyl-sphingomyelin, and N-methyldiethanolamine and other metabolites were present in the feces of CRC patients; while the abundance of acetate, propionate, butyrate, glucose, fructose, carnitine, glutamine, nicotinic acid, and linoleic acid were significantly decreased[53–57,67,68]. Concentrations of proline and cysteine, which are major components of most colonic epithelium mucus glycoproteins, also indicate significant changes in samples from CRC[57]. Palmitoyl-sphingomyelin, conjugated linoleate and p-aminobenzoate, which are metabolites of *Fusobacterium* and *Porphyromonas*, mediate CRC through postulated effects on cell shedding, inflammation and innate immunity[69]. Glutamate and succinate are intermediate products of glucose breakdown, and the increase in both levels may be associated with the upregulation of cancer glycolysis[53]. This weakness in cancer cells may be an effective drug target for the treatment of CRC[70].

Changes in blood metabolites

The serum metabolic profile has great potential in the early screening of CRC. Tricarboxylic acid cycling, urea cycling, glycolysis, arginine and proline metabolism, fatty acid metabolism and changes in polyamide metabolism were observed in serum or plasma metabolite studies[42,52]. Compared with healthy controls, an increase in 3-hydroxybutyric acid, formic acid, acetic acid, phenylalanine, proline, phosphoenolpyruvate, succinic acid, 1-methylguanosine, lipid, and glycoprotein is present in blood samples obtained from CRC patients, while the abundance of citrate, pyruvate, alanine, tyrosine, valine, creatine, glutamine, threonine, deoxyglucose, ribitol, ornithine, histidine, serine, tryptophan, lactic acid, hydroxylated polyunsaturated ultra long-chain fatty acids, and lysophosphatidylcholine is significantly decreased[31–49,51,52,71]. Among these, 3-hydroxybutyric acid, formic acid, acetic acid and other SCFA, lysophosphatidylcholine and other lipids, tyrosine, valine, tryptophan, phenylalanine, and other amino acids are common metabolites in most studies.

In serum obtained from CRC patients, a consumption of several potential precursors of glucose in gluconeogenesis, such as lactate, pyruvate, alanine, glutamine and other gluconeogenic amino acids, has been found to have no significant changes in glucose concentration compared with healthy controls. The observed trend may suggest the increased uptake of glucose precursors by the liver, which could be consistent with the increase in hepatic gluconeogenesis[46]. On the basis of these findings, TCA cycle changes may involve tumor growth in aggressive cancer cell proliferation, which requires large amounts of energy, and may lead to changes in the levels of some TCA cycle-related molecules[47]. The reduction of polyunsaturated long-chain fatty acids and the increase in long-chain fatty acids are associated with changes in fatty acid desaturases in CRC patients[50]. The vast majority of studies have confirmed that high serum levels of 3-hydroxybutyrate are present in patients with CRC. This molecule is formed during fatty acid oxidation, and its increased levels may suggest increased hepatic gluconeogenesis[46]. In addition to 3-hydroxybutyrate, it has been reported that the reduction in LysoPCs is associated with weight loss and the activation of inflammatory states in cancer patients. Thus, an observed decrease in LysoPCs in CRC patients may indicate a higher rate of decomposition of LysoPCs to support cancer metabolism and activities. The increased degradation of LysoPCs may lead to elevated levels of fatty acids[42].

Early screening of CRC

Diagnostic value of metabolites in CRC

Some studies have identified the metabolites of fecal, serum/plasma and characteristic metabolites of intestinal flora in

patients with CRC; and the sensitivity, specificity, accuracy and area under the receiver operating characteristic curve (AUC) were evaluated as a method of early screening for CRC. Together with NMR spectroscopy, 4 separation techniques coupled to mass spectrometry (MS) have been applied in recent metabolomics studies, that is, gas chromatography (GC), liquid chromatography (LC), capillary electrophoresis, and Fourier transform ion cyclotron resonance (FTICR)[27]. Different complementarity platforms are used for the detection of different metabolomics. At present, NMR has been gradually substituted by GC-MS and LC-MS, and GC-MS is a bit more versatile. In the LC-MS, UPLC-QTOF/MS is mainly used as nontargeted metabolomics, and UPLC-MS/MS is mainly used as target quantitative metabolomics. In addition, metabolites detected by tandem MS such as FIA-MS/MS, triple-quadrupole multiple reaction monitoring (TQ-MRM), and ESI-FTICR-MS seem more accurate. However, these expensive instruments are primarily used for research rather than clinic diagnosis. Zhu et al[35] established a partial least squares-discriminant analysis model using a panel of 5 metabolites (succinate, N2, N2-dimethylguanosine, adenine, citraconic acid, and 1-methylguanosine), and excellent model performance (sensitivity = 0.83, specificity = 0.94, and AUC = 0.91) was obtained, which was superior to the traditional CRC monitoring biomarker carcinoembryonic antigen (sensitivity = 0.75, specificity = 0.76, and AUROC = 0.80). Ritchie et al screened out hydroxylated polyunsaturated ultra long-chain fatty acids as metabolic biomarkers in all 3 independent cohorts of CRC patient samples using Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS). The targeted high-throughput TQ-MRM method successfully validated the FTICR-MS results in 2 further independent studies. The resultant receiver-operator characteristic curve AUCs ranged from 0.85 to 0.98 (average = 0.91 ± 0.04)[71]. Ikeda and colleagues constructed a model for evaluating metabolomic biomarker candidates including L-alanine, glucuronoic lactone and L-glutamine using multiple classification analysis. The sensitivity/specificity for these 3 metabolites was 54.5%/91.6%, 75%/75%, and 81.8%/66.7%, respectively[47]. Farshidfar and colleagues screened out a set of significant serum metabolomic profiles from various stages of CRC (n = 320) and healthy matched controls (n = 254). The sensitivity, specificity, accuracy (positive predictive value) and AUC of the diagnostic model was 85%, 86%, 89%, and 0.91 (95% CI, 0.87–0.96), respectively[34]. Lee and colleagues constructed a LMI discriminant equation (LOME) to investigate whether systematic LMI profiling might be applied to cancer screening. CRC LOME revealed excellent discrimination in a validation set (sensitivity = 93.21%, specificity = 96.47%). In addition, in a FOBT of available validation samples, the discrimination rate of CRC LOME was much stronger (sensitivity = 94.79%, specificity = 97.96%) than that of the FOBT (sensitivity = 50.00%, specificity = 100.0%), which is the standard CRC screening tool[41]. In another study, the predictive accuracy of the ^{1}H-NMR OPLS-DA model constructed by Amiot and colleagues for predicting CRC was also higher than FOBT (0.71; 95% CI, 0.56–0.83; $P = 0.0001$). The AUC of the diagnostic model and FOBT was 0.94 and 0.81, respectively[54]. The metabolites that revealed significant differences between early CRC patients and healthy controls were identified as a diagnostic model by Lin and colleagues. Among these, the sensitivity, specificity, accuracy, and area under the curve for butyrate at a threshold of 0.90 ppm was 69.2%, 84.6%, 76.9%, and 0.843 (95% CI, 0.692–0.995), respectively; while at a threshold of 1.56 ppm, the values were 72.1%, 92.3%, 80.7%, and 0.828 (95% CI, 0.666–0.991), respectively. For acetate, the values were 94.7%, 92.3%, 93.6% and 0.985 (95% CI: 0.949-1.021), respectively[53]. Nishiumi and colleagues analyzed the metabolomic data of 282 CRC patients and 291 healthy volunteers using GC/TQ-MRM, and established a stage 0/I/II CRC prognosis model. The area under the curve, sensitivity, and specificity values of this model for detecting stage 0/I/II CRC was 0.996, 99.3%, and 93.8%, respectively. The sensitivity and specificity of the model to each disease stage were > 90%. Surprisingly, the sensitivity for stage 0, specificity for stage 0, and sensitivity for stage II of the disease were all 100%[44]. Therefore, metabolites, as a means of early screening CRC, have good clinical value.

Limitations

Despite the large number of reports of fecal and blood metabolites in CRC in recent years, it is still necessary to realize the limitations of these studies: (1) relative small sample size and population heterogeneity. At present, the number of samples of blood and fecal metabolomics for CRC patients is generally low, and there are large variations in the results of different studies. Moreover, there are inevitably heterogeneity in the population involved in this study. It is a tremendously complex topic, as microbiota vary considerably from one individual to another, and vary in one individual along their life course. Therefore, multicenter, multipopulation and large sample studies are needed to enhance the repeatability of the identified candidate biomarkers. (2) Detection techniques. At present, there is still a lack of recognized standards for the detection of metabolites, and there are large individual differences between various researches and platforms. There are also problems in quality control. Furthermore, most researches used a single analytical technology platform, since the existing analytical technology platform does not cover all kinds of metabolites. Therefore, only a small number of metabolites were covered. (3) Statistical methods. Since samples in vivo and in vitro are affected by a variety of confounding factors, there are obviously a number of biases when applying statistical methods; and the present CRC study does not adequately remove these confounding factors, thereby affecting the reliability of the results. The use of multiple machine algorithms to carry out the construction of the early diagnosis model is also an important part of the present study. (4) Limitations of diagnostic tests. Most studies lack the sensitivity and specificity of diagnostic tests, and some biomarkers screened by these diagnostic tests even revealed opposite trends in different studies. (5) Lack of systematic approach. The problem with our approach to the analysis of CRC, or any physiological or disease process for that matter, is that we apply a reductionist approach. We look at individual molecules, genes, or in this article's case, metabolites. In this way, we are largely influenced by our own biases, which may partially explain the fact that a given biomarker may be higher in one study of CRC but lower in another. A systems approach, analyzing thousands upon thousands of biomarkers in systematic, unbiased manner using appropriate modelling techniques and statistics will not only provide us with relevant and clinically significant biomarkers, but also enable us to compare the importance of each biomarker relatively.

The direction of future research

At present, metabolomics has recently become a focus in researches, and its application in various fields is rapidly growing. However, the metabolic research of CRC remains in its development stage. Hence, there is an urgent need for larger sample size and comprehensive cohort studies of multiethnic and regional populations. These analytical techniques remain limited. At the same time, technologies capable of measuring multiple metabolites and a comprehensive database that can integrate large data from different studies are urgently needed. Early diagnosis is essential for CRC management, and blood and fecal metabolomics have been proven as promising diagnostic biomarkers. Therefore, further studies are warranted for the early screening and feasible diagnostic practice targeting of these metabolomics.

Ethical approval

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Author contribution

C.K.: wrote the manuscript; R.G. and C.K.: retrieved the literatures; X.Y. and H.Q.: supervised the manuscript.

Conflict of interest disclosure

The authors declare that they have no financial conflict of interest with regard to the content of this report.

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