

Chapter 5

Metabolic Markers for Early Detection of Gastrointestinal Cancers



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Abstract Gastrointestinal cancers are a major threat to humans nowadays, due to their high incidence and mortality rates. The early detection of GI cancers was the key to prevention and treatment. Metabolomic profiling and analysis of volatile biomarkers are new and promising screening tools for the diagnosis of several cancers. Metabolites are small molecules produced during cell metabolism that represent the functional status of cell/tissue/malignant phenotype. Studies on biofluid metabolomics (serum, urine, plasma) are emerging fast on detecting new biomarkers for early diagnosis of cancer diseases and grasp a great promise for diagnostic applications. Volatile Organic Compounds (VOCs) signify diverse volatile metabolites which can be able to emit and diagnose in urine, breath, sweat, and feces. Nowadays, there is an increasing interest in the use and evaluation of VOCs with emerging analytical technologies in the diagnosis of GI cancer. With the advent of novel technologies in clinical diagnosis, metabolome analysis is an effective tool for metabolite profiling of biological processes in cells/tissues. This review emphasizes recent advancements in the identification of GI cancer biomarkers, particularly it focuses on metabolic markers and the emerging field of volatile biomarkers.

Keywords Gastrointestinal cancers · Metabolomic profiling · Volatile biomarkers · Clinical diagnosis

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5.1 Introduction

Gastrointestinal (GI) cancers are malignant with high mortality rates in the GI tract and accessory organs of digestion (Tillu and Nagaraju 2017). GI malignancies account for 30% of all cancer incidence and represent approximately 40% of tumor-related mortality globally. However, GI cancers are declining worldwide; however, it is even higher in Eastern Asia, mainly in China and Japan (Prathyusha et al. 2017). GI cancers have a high rate of mortality and morbidity due to a lack of defined risk factors, delayed diagnoses in their early stages leading to the progression of advanced stages with high recurrence (Tillu and Nagaraju 2017). Diagnosis in earlier stages considerably increases the prognosis by 95% in GI cancer patients (Xu et al. 2013). Esophagus, pancreatic, colorectal cancer (CRC), and gastric adenocarcinoma are among the top GI cancers in both incidence and mortality.

Several diagnostic tests are available for screening cancer, viz. upper and lower GI endoscopy-biopsy with pathological evaluation of tissue (Correa 2013; Thrumurthy et al. 2013), non-endoscopy-based balloon cytology, barium esophagram (Groome et al. 2008), image-based tests such as CT (computerized tomography) scan, Cytosponge, MRI (magnetic resonance imaging), cystoscopy, sigmoidoscopy, colonoscopy (Wild et al. 2010) and serological markers (Zhang et al. 2013, Patel and Ahmed (2015). Carcinoembryonic antigen (CEA) and fecal occult blood testing (FOBT) are currently in use for diagnosis of CRC with relatively low sensitivities and specificities (Ransohoff and Sox 2016). Nonetheless, all pragmatic approaches possess specific limitations in diagnosing imaging techniques. A major drawback is in some cases cancers endure undetected until malignant stages, in turn, affect the survival rate. The accuracy of endoscopy-biopsy screening and examination depends on collecting appropriate tissue biopsy which is a common clinical practice while a battery of serological marker tests was unsuccessful for screening or surveillance because of their low accuracy, sensitivity, and specificity. Many biopsy techniques are deliberate to be uncomfortable, inconvenient to patients, and are often underutilized by large segments of the population for a variety of reasons, such as access and patient burden (Oakley-Girvan and Davis 2017). Nonetheless, this necessitates the requirement for cost-effective, sensitive, reliable, accurate, noninvasive, patient-friendly screening tools for GI cancer detection which are currently not employed. Ascertaining specific biomarkers for detection of GI cancer in the benign stage (at onset) to ensure patient survival seems very promising.

Metabolites are small molecules produced during cell metabolism that represent the functional status of cell/tissue/malignant phenotype. The uncontrolled proliferation and altered metabolisms (glycolysis, TCA cycle, choline, and fatty acid metabolism) are important hallmarks of the malignant phenotype. These altered metabolisms result in a metabolic landscape that diverges significantly among cancer and normal cells. The goal of metabolomics studies captures this unique metabolic portrait as a label of cancer. Furthermore, a wide range of metabolic by-products is produced, comprising of a battery of organic compounds characterized by volatility at body temperatures, thus known as volatile organic metabolites/compounds

(VOCs). Many VOCs are constantly produced in the body during various molecular pathways. VOCs are low molecular weight and volatile compounds detectable at low concentrations in biofluids, feces, and exhaled breath. Therefore, metabolites and VOCs can be highly informative regarding cell/tissue phenotype (Broza et al. 2018). Studies on biofluid metabolomics (serum, urine, plasma) and VOCs are emerging fast on detecting new biomarkers for early diagnosis of cancer diseases and grasp a great promise for diagnostic applications (Buck et al. 2017). Undoubtedly, several studies reported that specific regions of NMR and MS spectra differ among cancer patients and healthy controls (Patel and Ahmed 2015).

5.2 Metabolomic Analyses

Metabolic profiling involves two principal analytical procedures, viz. nuclear magnetic resonance (NMR) (Wang et al. 2017a, b) and mass spectrometry (MS) (Dettmer et al. 2007). Variations of NMR include high-resolution magic angle spinning (HR-MAS NMR) intended for tissue samples, ¹H-NMR, 2D-NMR (HSQC and HMQC) techniques have been widely used in NMR-based metabolomics marker study (Emwas 2015). The chemical and physical diversity of molecules in biofluids, and alterations in ionic strength, pH, temperature, etc. may impede NMR analysis (Zhang et al. 2013). MS may be coupled directly to a chromatographic column such as gas chromatography-mass spectroscopy (GC-MS), liquid chromatography-mass spectroscopy (LC-MS), or capillary electrophoresis (CE-MS) (García et al. 2017) based on appliance. However, LC-MS is further ramified into ultra-performance mass spectroscopy (UPLC-MS) (Chen et al. 2019) or high-performance mass spectroscopy (HPLC-MS) (Zhao et al. 2006), and LC/MS/MS (liquid chromatography with tandem mass spectrometry). MS analysis can be performed using different ionization methods and mass analyzers (quadrupole time-of-flight (Q-TOF), Fourier transform ion cyclotron resonance (FTICR), and Orbitrap) for separation of ions of different masses and detection of molecular fragments based on mass-to-charge ratios (m/z). HPLC, coupled with electrospray ionization and Q-TOF (HPLC/ESI/Q-TOF-MS), was widely in use and documented to be beneficial clinically for early diagnosis of GI cancer due to precise qualitative analysis (Callejón-Leblic et al. 2016; Wang et al. 2017a, b).

Metabolome analysis was categorized into three major ways: targeted analysis, metabolic fingerprinting, and metabolite profiling (untargeted analysis). Untargeted analysis (metabolic profiling) usually emphasizes the measurement of levels of metabolites of all detectable metabolites in a given sample. NMR technique is widely in use for metabolomics fingerprinting (Emwas 2015). Metabolic fingerprinting reflects total metabolite fingerprint as a rare pattern illustrating a glimpse of the particular cell line or tissue metabolism. The targeted analysis emphasizes on the quantification and documentation of metabolites, of a specific metabolic pathway or direct product of drug administering or food intake. The metabolites involved in the study are usually predetermined, in targeted analysis, and the sample preparation is

modified to diminish interference from other metabolites (Zhang et al. 2013). Djukovic and group employed targeted metabolite procedure to examine the role of nucleosides as cancer biomarkers in EAC (Esophageal Adenocarcinoma) using a serum-NMR technique (Djukovic et al. 2010).

Two main approaches have been developed that are used to assess VOCs in biological samples. Identification of the VOC spectrum allows one to study each compound separately and estimate the likelihood that it can serve as an individual biomarker of disease. One approach is based on analytical techniques such as GC-MS (Altomare et al. 2013), proton transfer reaction mass spectrometry (PTR-MS) (Jordan et al. 1995) and selected ion flow tube mass spectrometry (SIFT-MS) (Krilaviciute et al. 2015). Furthermore, the second approach is based on diverse chemically sensitive gas sensors that provide a total assessment of the mixture/profile of VOCs in a sample (Lourenço and Turner 2014).

Complex metabolite profiles obtained after analytical techniques were interpreted and characterized by multivariate pattern recognition statistical analysis, viz. (1) principle component analysis (PCA) (Wang et al. 2018), consensus PCA (CPCA), partial least square discriminant analysis (PLS-DA) (Nakajima et al. 2018), orthogonal signal correction (OSC), orthogonal partial least square discriminant analysis (OPLS-DA) (Li et al. 2013), and graded clustering analysis (GCA) (Wishart et al. 2016; Lai et al. 2018). XCMS is the most commonly used tool for metabolite profiling to analyze MS data by matching and nonlinear peak alignment. SIMCA-P is employed for multivariate data analysis (MVDA) by PLS, OPLS, and PCA analysis. MZmine 2 is a modular framework for visualizing, analyzing, and processing MS data. MetaboAnalyst is a web-based pipeline for statistical analysis, metabolomic data processing, and consequent functional elucidation. Notably, several additional tools also help in spectrometric data analysis. Davis et al. employed Mann–Whitney statistical analysis tool to correlate individual metabolite concentration among controls and EC patients (Davis et al. 2012).

5.3 Serum Metabolomics Signatures

Digestive tract malignant neoplasms are the most common reason for cancer-related mortality globally. Ikeda and his co-workers reported more sensitive metabolites for diagnosis of GI cancer using analytical tools LC-MS, GC-MS, and NMR followed by PCA and PLS-DA statistical analysis. Their studies reported the metabolic differences among esophagus, gastric, and CRC cancer signifying the importance of different metabolisms in these cancers. Perhaps, these studies using multiple classifications analysis identify metabolite alteration levels namely malonic acid and L-serine is characteristic for EC. Alterations in concentrations of pyruvic acid and 3-hydroxy propionic acid is characteristic for gastric cancer while L-glutamine and glucuronic lactone signify colorectal cancer (Ikeda et al. 2012).

5.3.1 *Esophagus and Stomach*

Esophagus cancers are extremely malignant affecting the upper digestive tract with a 5-year survival rate in less than 15%. Zhang and colleagues (2013) analyzed the serum metabolic signatures of EC patients with healthy control using ¹H NMR and UPLC based focused metabolic profiling. EC cells exhibited significant perturbations in glucose (reduced glucose and increased lactate levels in EC patients), lipids (apolipoproteins, LDL, VLDL, and unsaturated lipid were considerably reduced in the serum of EC patients), energy (serum creatinine and creatine concentrations were considerably elevated among EC patients), and amino acid metabolism. However, 12 biomarkers using NMR and 7 markers from UHPLC focused metabolomics were identified. Strikingly, the ketone bodies, viz. acetoacetate and β -hydroxybutyrate level were specifically elevated in EC patients' serum suggesting a promoted β -oxidation. Amino acids (L-Tryptophan, L-Tyrosine, linoleic acid, and palmitic acid) have been identified to be modified in ESCC (Zhang et al. 2013). Wang et al. reported serum metabolites for the early-stage detection and discrimination among different stages of esophageal squamous cell carcinoma (ESCC) using nonparametric Kruskal–Wallis rank-sum test. Metabolites of glycerophospholipid, linoleic acid, and choline metabolism were to be found dysregulated in the ESCC population assessed to healthy individuals. Decreased trends of three biomarkers, namely dodecanoic acid, lysophosphatidic acid (LPA), and 4-lysophosphatidylcholines (LysoPC), reported being clear hallmarks for ESCC progression (Wang et al. 2016).

5.3.2 *Colorectal Cancer (CRC)*

CRC is one of the most prevailing cancer types globally with high morbidity and mortality. Uchiyama et al. reported serum metabolites for early-stage detection. Metabolome analysis was performed by CE-TOFMS for serum samples of CRC patients and controls followed by HCA and PCA statistical analysis and detected metabolites were plotted using VANTED software. Seventeen metabolites were reported to be correlated with CRC by elevation and 16 by downregulation in patients with adenoma in comparison with controls. Clustering identified upregulation of 7 and downregulation of 29 metabolites in stage I CRC, while upregulation of 11 and downregulation of 19 metabolites in stage II CRC. In stage III CRC, 5 metabolites reported to be upregulated whereas 39 downregulated; and in stage IV, 6 metabolites reported to be upregulated and 28 downregulated compared to normal controls. 3-hydroxybutyric acid was elevated in CRC stages and isovaleric acid, ornithine, benzoic acid, and the amino acids His, Lys, and Trp were downregulated. Of these, decanoic acid, octanoic acid (upregulated), histidine (downregulated), and benzoic acid exhibit significant correlation with all CRC stages. Benzoic acid is one of the metabolites that originated from procyanidins

degradation by human gut microbiota. Benzoic acid was reported to be an excellent diagnostic marker to detect early and all the stages of CRC (Uchiyama et al. 2017). Hata and his co-workers reported a promising serum biomarker GTA-446 (gastro-intestinal tract acids) for primary CRC screening with the aim of early detection and to detect peoples at higher risk of CRC (Hata et al. 2017).

5.4 Plasma Metabolic Signatures

Plasma free amino acids (PFAA) are one of the most essential compounds for focused metabolomics as they play important physiological roles as elementary metabolites and metabolic regulators. Several studies reported that amino-acid levels were drastically declined in early-stage cancer populations, irrespective of subsequent progression. Particularly, significant reductions in the concentration of all amino acids were detected in both GC and CRC patients. Glutamine, tryptophan, histidine, proline, and ornithine amino acids were related to all types of cancer unveiled by univariate analysis (Miyagi et al. 2011).

5.4.1 Colorectal Cancer (CRC)

Cancers are characterized by diverse metabolic phenotypes due to alterations in key metabolic pathways, viz. glycolysis or tricarboxylic acid (TCA) cycle, etc. Geijsen et al. (2019) reported the correlation between plasma metabolites and colorectal cancer. Wang et al. reported a plasma metabolite marker for CRC diagnosis using a two-stage case-control study employing UPLC/Q-TOF MS/MS and PCA analysis. Glycochenodeoxycholate, L-Tryptophan, 13-OxoODE, IDP, and LysoPC (16:0), a five-biomarker panel, exhibited excellent diagnostic performance, favorable biological significance, and tumor specificity in Northeast China population. A six-biomarker panel of L-Phenylalanine, Linoleic acid, Citric acid, Inosine, Glycocholic acid, and LysoPC (14:0) showed the best extrapolation in diverse populations. Four metabolites (L-Tryptophan, Linoleic acid, Glycocholic acid, and LysoPC (16:0)) were ultimately recommended as the best combination with multiple advantages (Wang et al. 2018).

5.4.2 Esophagus and Stomach

In recent years, metabolomics studies on EC were performed by diverse analytical techniques to discover potential therapeutic markers. Liu et al. reported the metabolic profiling of ESCC by using UPLC-ESI-TOFMS and identified six upregulated molecules, namely phosphatidic acid, phosphatidylserine, phosphatidylethanolamine phosphatidylinositol, sphinganine 1-phosphate, and phosphatidylcholine.

Furthermore, bile acid, lithocholic acid taurine conjugate reported being significantly upregulated whereas 5- β -cyprinol sulfate and desmosine/isodesmosine are downregulated in the plasma of ESCC patients (Liu et al. 2013).

5.5 Urinary and Fecal Metabolic Signatures

Urine is a biofluid usually used by metabolomics scientists, due to the collection of large volumes and patient-friendly.

5.5.1 Colorectal Cancer

Urine metabolic profiles were analyzed by Wang and his co-workers using ^1H NMR along with OPLS-DA statistical validation by permutation analysis in comparison with healthy controls at stage III. They reported perturbation in amino acid (asparagine, alanine, cysteine, and phenylalanine), glycolysis, TCA cycle, choline (acetoacetate, guanidinoacetate), vitamin-B3 (Trigonelline), and urea metabolisms. Metabolites upregulated and downregulated at different stages were summarized in Table 5.1 (Wang et al. 2017a, b). Le Gall and his research group quantified fecal extracts from CRC patients and screened over 80 molecules using NMR. Furthermore, isobutyrate, branched-chain fatty acids (BCFA), valerate, isovalerate, and phenylacetate were reported to be upregulated while reduced concentrations of amino acids, methanol, sugars, and bile acids (lithodeoxycholate, cholate, and deoxycholate) in the fecal extracts of ESCC patients (Le Gall et al. 2019).

5.5.2 Esophagus and Stomach

Altered carbohydrates, amino acids, lipid, and ketone metabolisms were reported to be hallmarks for EC. Xu and his research group reported global urine metabolic profile of ESCC patients and healthy controls using LC-MS along with MVDA and reported 19 potential biomarkers related to perturbations of amino acids fatty acid β -oxidation and nucleotide metabolism. Metabolites upregulated and downregulated at different stages were summarized in Table 5.1 (Xu et al. 2016). Recently, studies are employing metabolomics technology to tissue, plasma, serum, and urine samples revealed alterations in choline, glucose, amino acid, fatty acid, linoleic acid, and energy metabolism in EC cells. These metabolites upregulation and downregulation reflected in blood samples of patients indicates the importance of these metabolisms in esophagus cancer progression and early detection.

Table 5.1 An overview of study design, analytical techniques, identified compounds for early detection of GI cancers

S. no	Cancer type	Analytical platform	Sample type	Statistical analysis	Metabolites of cancer compared to control		Biomarker	References
					Upregulated	Downregulated		
1.	CRC	CE-TOFMS	Serum		Decanoic acid Octanoic acid	Histidine, Benzoic acid (biomarker for early CRC)	Benzoic acid	Uchiyama et al. (2017)
2.	CRC	¹ H NMR	Fecal water extracts	Robust PCA	Cysteine, proline	Acetate and butyrate		Monleon et al. (2009)
3.	CRC	UPLC/Q-TOF-MS/MS	Plasma	PCA	Phosphatidylcholines, linoleic acid, gamma-linolenic acid, 13-L-hydroperoxylinoleic acid, 13S-hydroxyoctadecadienoic acid, and 13-OxoODE	2-Aminobenzoic acid, 13-OxoODE, Citric acid, 2'-Deoxyinosine triphosphate, Taurocholic acid, and LysoPC	L-Tryptophan, Linoleic acid, Glycocholic acid, and LysoPC	Wang et al. (2018)
4.	CRC	¹ H NMR	Urine	OPLS-DA	Thymidine, Fumarate, Hippurate, Cis-Aconitate, Pyridoxic acid, Cinnamic acid, Homogentisic acid, Indoleacetate, Trigonelline	Creatine, Creatinine, Uracil, Urea		Wang et al. (2017a, b)
5.	Esophageal cancer	NMR-based metabolomics and UHPLC-based focused metabolomics	Blood serum	OPLS-DA, PCA	β-Hydroxybutyrate acetoacetate Creatine Creatinine, Lactate, Glutamate, Glutamine, Histidine	LDL/VLDL, Unsaturated lipids, Acetate, Glucose, Tyrosine		Zhang et al. (2013)
6.	ESCC	UHPLC-QTOF/MS	Blood serum	PCA, PLS-DA		Dodecanoic acid, lysophosphatidic acid (LPA), and 4-lysophosphatidylcholines (LysoPC)		Wang et al. (2016)

7.	ESCC	¹ H NMR	Serum	PCA, PLS-DA				α-Glucose, choline, glutamine, glutamate, valine, and dihydrothymine	Yang et al. (2019)
8.	Esophageal cancer	¹ H NMR	Tissue	OPLS-DA, PCA	Acetate, short-chain fatty acid and GABA, creatinine, creatinine, DMG, DMA, and TMA	Glucose, AMP and NAD, upregulation of formate			Wang et al. (2013)
9.	ESCC	UPLC/TOF/MS	Plasma	PCA	Phospholipids, sphinganine 1-phosphate, bile acid, lithocholic acid taurine conjugate	Desmosine/isodesmosine and 5-β-cyprinol sulfate			Liu et al. (2013)
10.	GI cancer	LC/MS, NMR, GC/MS	Serum	PCA, PLS-DA				Malonic acid and L-serine -esophageal cancer 3-hydroxypropionic acid and pyruvic acid- gastric cancer L-alanine, glucuronic lactone and L-glutamine- and L-glutamine- CRC	Ikeda et al. (2012)
11.	ESCC	LC-MS/MS, NMR	Urine	OPLS-DA	Pyroglutamic acid, Indoxyl, Uroacanic acid, L-Carnitine, L-Fucose, Uric acid, Acetylcarbitine, Deoxycytidine, cAMP, cGMP, Phenylacetylglutamine	Paraxanthine, Heptanoylcarbitine, Octenoylcarbitine., Nonenoylcarbitine, Nonanoylcarbitine, Decanoylcarbitine, Undecenoylcarbitine, Undecanoylcarbitine			Xu et al. (2016)

(continued)

Table 5.1 (continued)

S. no	Cancer type	Analytical platform	Sample type	Statistical analysis	Metabolites of cancer compared to control		Biomarker	References
					Upregulated	Downregulated		
12.	CRC	MRB-CE-ESI-MS	Urine		Isoleucine, valine, arginine, lactate acid, and leucine	Histidine, methionine, serine, aspartic acid, citric acid, succinate, and malic acid	GTA-446	Chen et al. (2012)
13.	CRC	LS-MS/MS	Serum			GTA-446	GTA-446	Hata et al. (2017)
14.	CRC	RRLC-TOF/MS	Urine	PCA, PLS-DA		Dihydrospingosine, Sphinganine, O-octanoyl-L-carnitine		Yue et al. (2013)
15.	CRC	¹ H NMR	Fecal extracts		Branched-chain fatty acids (BCFA), isovalerate and isobutyrate, valerate, and phenylacetate	Amino acids, sugars, methanol and bile acids (deoxycholate, lithodeoxycholate, and cholate		Le Gall et al. (2019)
16.	CRC	LC-MS	Urine	PLS-DA			Polyamines	Nakajima et al. (2018)
17.	CRC	DI-ESI-FTICR MS ^a	Serum	OPLS-DA			Palmitic amide, oleamide, hexadecanedioic acid, octadecanoic acid, eicosatrienoic acid, LPC (18:2), LPC (20:4), LPC (22:6), myristic acid, and LPC (16:0)	Li et al. (2013)

5.6 Volatile Organic Compounds

VOCs are a diverse group of carbon compounds emitted from the human body that reflects the metabolic condition of the person detected in blood, breath, and excreted body fluids. Hundreds of different VOCs have been reported in cancer patients from different sources. Acids, ketones, alcohols, amines, aldehydes, O-heterocycles, N-heterocycles, VOCs, and other VSCs have been detected and quantified. However, concentrations of specific organic compounds in the exhaled breath of cancer patients are thought to be the result of oxidative stress, abnormal metabolic processes, or inability of biological systems to detoxify ROS (reactive oxygen species). Some VOCs are thought to originate from cancer cells as a result of abnormal metabolism that does not exist in healthy people (Oakley-Girvan and Davis 2017). Among several extraction procedures SPME (solid-phase microextraction) is a prevailing technique for sample preparation, sampling a wide range of analytes in breath and biological samples (Kim et al. 2019).

The four studies targeting to identify VOC in breath samples of CRC patients suggested diverse patterns of potential biomarkers were not completely matched, with an exemption of 1,3-dimethyl benzene reported by both Peng and Altomare groups individually (Peng et al. 2010; Altomare et al. 2013). Furthermore, 4-methyl octane was reported as a potential biomarker both by Altomare and Amal groups individually (Altomare et al. 2013; Amal et al. 2016). These diverse patterns possibly due to the involvement of different methods in sample collection and analysis. Wang et al. examined two different samples, of CRC patients i.e., exhaled breath and blood using SPME-GC-MS technique. Two different VOC patterns that did not fit each other probably due to different sample characteristics are reported. Remarkably, these authors identified reduced levels of 6-t-butyl-2,2,9,9-tetramethyl-3,5-decadien-7-yne in breath and blood sample. Kumar and his research group analyzed breath samples from 81 esophageal or gastric adenocarcinoma patients against 129 controls including benign upper gastrointestinal diseases, Barrett's metaplasia, and normal upper GI tract. Twelve VOCs—hexanoic acid, pentanoic acid, methyl phenol, ethyl phenol, phenol, butanal, hexanal, pentanal, octanal, heptanal, decanal, and nonanal, were reported at extremely high levels in cancer groups compared to controls (Kumar et al. 2015). Kim and his co-workers employed SPME and 2D GC-MS to analyze 30 random plasma samples from CRC patients and reported five VOCs, among them 2,3,4-trimethylhexane (reduced) and 2,4-dimethylhept-1-ene (enhanced) were both lipid peroxidation products (Kim et al. 2019). Wang and his co-workers analyzed blood VOCs of SW620 CRC mice for a course of 12 and 26 days. They reported eight VOCs which progressively augmented with tumor growth (acetone, glycerol, arsenous acid, 2-heptanone, 4-nonanone, tris (trimethylsilyl) ester, butylated hydroxytoluene, l-alanine ethylamide (S)-, and 3-heptanone,4-methyl 2-dodecanone). The concentration of VOCs peaked at maximum tumor size and decreased promptly after tumor resection. Cyclotrisiloxane, hexamethyl was utilized by the tumor, and its concentration progressively reduced with the size of the tumor (Wang et al. 2019).

5.7 Nanomaterial-Based Breath Tests

Nanomaterial-based breath tests were first developed by Haick and co-workers that could categorize among different cancer types in exhaled breath, regardless of patients' lifestyle, habits, gender, and other confounding factors. Xu et al. used GC-MS for chemical analysis of breath samples reported five VOCs (2-butoxyethanol, 2-propenenitrile, furfural, isoprene, and 6-methyl-5-hepten-2-one) elevated in gastric cancer and gastric ulcer patients. Three different DFA models were developed to achieve excellent discrimination among them (1) GC vs. benign gastric conditions, reported with 89% sensitivity; 90% specificity; (2) different stages of GC early-stage (I and II) and late-stage (III and IV), with 89% sensitivity; 94% specificity (Xu et al. 2013). Tong et al. reported four nanomaterial-based biomarkers in gastric cancer patients vs. healthy individuals 1,3-Dioxolan-2-one, Undecane, 2,3-Butanediol, 3,8-dimethyl- *N,N*-Dimethylacetamide Hexadecane while (*p*-hydroxyphenyl), 1,3-Dioxolane-2-methanol and 3,5-Decadien-7-yne, Phosphonic acid, 6-*t*-butyl-2,2,9,9-tetramethyl-1,6-Dioxacyclododecane-7,12-dione for carcinoma patients vs. gastric ulcer patients (Tong et al. 2017).

5.8 Integration of Metabolomic Networks

In the human body, most cancer cells exhibit hypoxic environment due to increased cell division at early stages resulting in increased aerobic and anaerobic glycolysis which is accompanied by disrupted TCA cycle and even other metabolisms creating a mutagenic phenotype. Tumor cells predominantly generate energy through glycolysis rather than oxidative phosphorylation via the TCA cycle, even in aerobic conditions (Warburg effect) (Armitage and Southam 2016). This results in the production of lactate in large amounts by the tumor and the anabolic synthesis of nucleotides, amino acids, and lipids. Lactate produced in large amounts is converted to glucose in liver via Cori cycle ends in acetyl Co-A accumulation and citrate upregulation (disruption of TCA cycle). If acetyl-CoA is not well utilized in TCA cycle, ketogenesis takes place and thus the accumulation of ketone bodies, viz. acetoacetate and β -hydroxybutyrate (Zhang et al. 2013). Increased glucose utilization favors energy metabolism, NADPH recycling to maintain serum creatine, creatinine, and glutathione levels for an optimal cellular redox status, respectively (Wang et al. 2013). Many blood amino acids downregulated in cancer patients indicate an increased demand for and overutilization of amino acids in tumor tissue. Altered fatty acid metabolism is also reported in cancer patient's plasma, by reduced levels of several unsaturated lipids, VLDL, and LDL (Hasim et al. 2012). Wang et al. first reported the importance of essential fatty acids (linolenic acid) in proliferating tumor cells for the biosynthesis of prostaglandins and cell-membrane assembly and their metabolites as biomarkers (Wang et al. 2018). In summary, altered/disrupted metabolomic pathways comprise fluctuations in glycolysis, Cori cycle, TCA cycle,

an amino acid (Alanine, Leucine, Valine, Isoleucine, 1-methylhistidine, and Glyco-protein), a ketone body, fatty acid (unsaturated lipids, VLDL, and LDL), energy metabolisms, and urea cycle. Metabolites accumulate in these pathways represent typical metabolic and VOC signatures in GI cancer patients.

5.9 Conclusion and Future Directions

Currently available techniques for diagnosis and surveillance of GI cancers are expensive, invasive, and not suitable for early detection. Robust, low-cost, and noninvasive biomarkers to facilitate early screening, surveillance are scanty, indicating the necessity for the development of more efficient methods. In this sense, the metabolomics field emerged highly promising in the past few years, with large advancements in GI cancer diagnosis based on metabolites. Metabolic profiling has been used to find novel biomarkers for early detection of cancers. However, metabolomic analysis is a promising approach in cancer diagnosis, with certain constraints comprising necessity to evaluate existing metabolites, data redundancy, false discovery problems, and cost limitations endure major hurdles for metabolomics research. Issues related to sensitivity, specificity, and accuracy for biomarker detection must be dealt with to unleash metabolomics potential. Furthermore, the diagnostic accuracy of metabolic signatures needs to be established (Figs. 5.1 and 5.2).

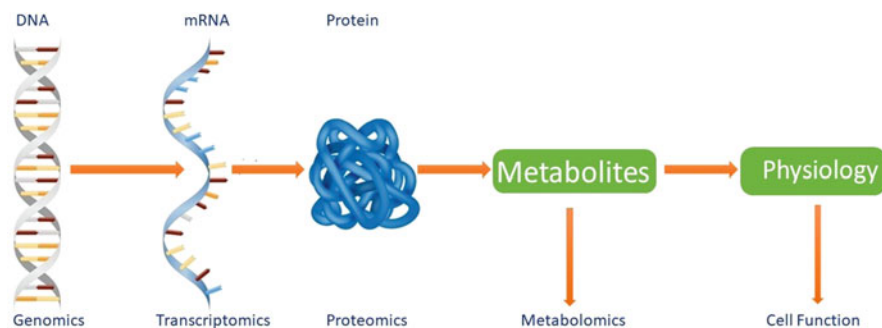
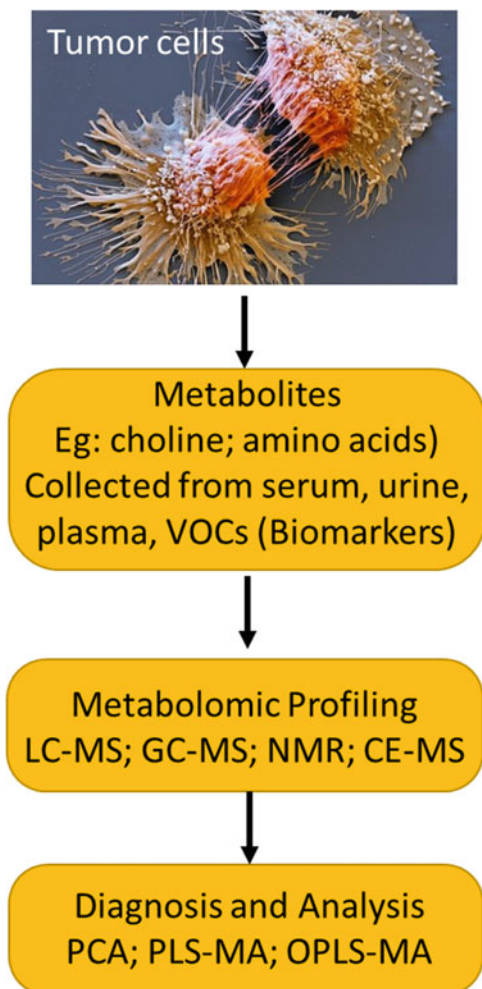


Fig. 5.1 Genomics to metabolomics

Fig. 5.2 Schematic representation of metabolome profiling



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