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Abstract: Cancer has myriad effects on metabolism that include both rewiring of intracellular metabolism to enable cancer cells to proliferate inappropriately and adapt to the tumor microenvironment, and changes in normal tissue metabolism. With the recognition that fluorodeoxyglucose-positron emission tomography imaging is an important tool for the management of many cancers, other metabolites in biological samples have been in the spotlight for cancer diagnosis, monitoring, and therapy. Metabolomics is the global analysis of small molecule metabolites that like other *-omics* technologies can provide critical information about the cancer state that are otherwise not apparent. Here, the authors review how cancer and cancer therapies interact with metabolism at the cellular and systemic levels. An overview of metabolomics is provided with a focus on currently available technologies and how they have been applied in the clinical and translational research setting. The authors also discuss how metabolomics could be further leveraged in the future to improve the management of patients with cancer.

Keywords: cancer, intracellular, metabolism, metabolomics

Introduction

In cancer cells, metabolism is dysregulated to support the demands of uncontrolled proliferation.[1-3] This rewiring of cellular metabolism leads to characteristic metabolic phenotypes that can be used for earlier cancer diagnosis, patient selection strategies for clinical trials, and/or as biomarkers of treatment response. Altered metabolism also results in unique metabolic dependencies that, in some cases, can be targeted with precision medicine and nutrition, including drugs that selectively target metabolic enzymes.[4,5] Cancer and cancer therapies can also alter metabolism at the whole-body level and interact with the metabolic effects of diet and exercise in complex ways that may affect cancer outcomes and impact a patient's quality of life.

In the past, much of the assessment of metabolic changes has been limited to measuring individual hormones and metabolites using imaging modalities and standard clinical laboratory tests. In contrast, metabolomics involves the systematic measurement of many metabolites, including nutrients, drugs, signaling mediators, and the metabolic products of these small molecules in blood, urine, tissue extracts, or other body fluids.[6] Metabolomics is a powerful tool that can identify cancer biomarkers and drivers of tumorigenesis.[7] The objective of this review is to provide an overview of current and future opportunities of metabolomics to improve the diagnosis, monitoring, and treatment of cancer. We begin with a review of how

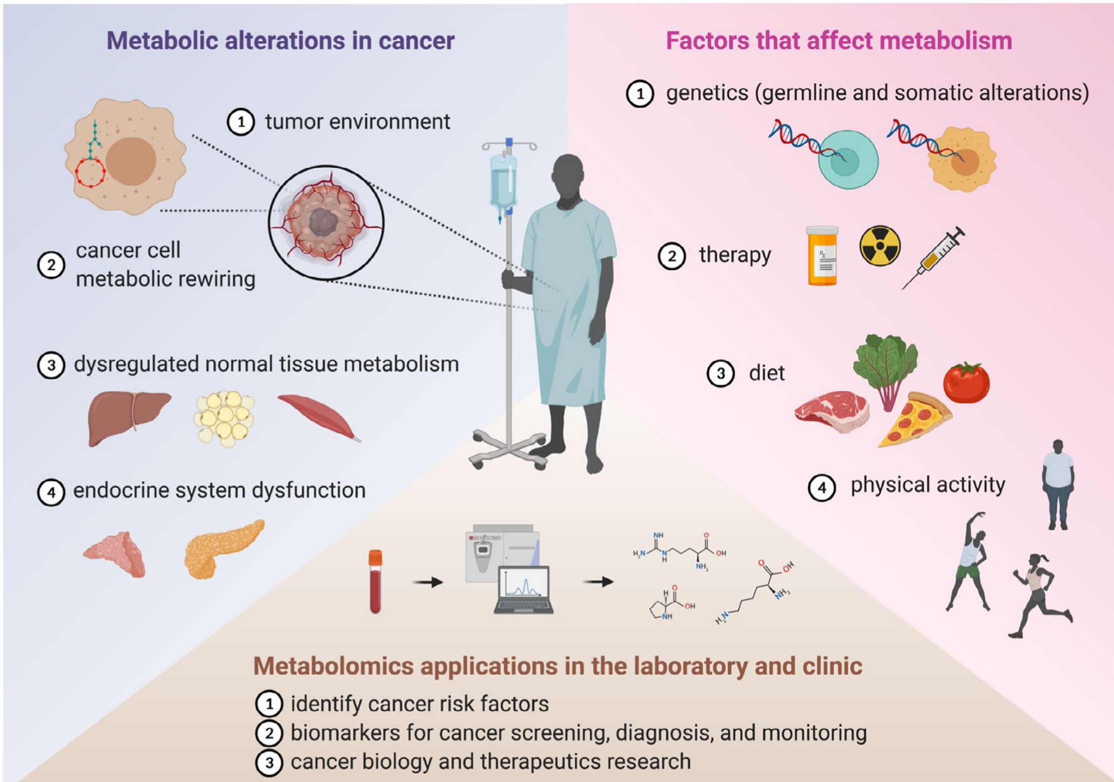

FIGURE 1. Cancer and metabolism interact at many levels. Cancer causes metabolic alterations in cancer cells and normal tissues, which, in turn, interact with intrinsic and extrinsic factors to affect systemic metabolism. Metabolomics is a systems-based approach used to define these complex metabolic interactions for diagnostic and therapeutic gain. See text for details.

metabolism and cancer interact at the level of cells, tissues, and the whole body (Fig. 1). We then introduce general technical aspects of metabolomics, instrumentation, and source material, including the pros and cons of different approaches. In the final section, we provide examples of how metabolomics has been used in the clinical and translational research setting as a way to guide potential future applications. Because the role of metabolism in cancer has been extensively covered, the reader is referred to other excellent reviews cited throughout this article for a more in-depth discussion of specific topics.

Metabolism in Tumors and Patients With Cancer

In this section, we explore the intersection between metabolism, cancer, and therapy. We begin with a discussion of metabolic alterations in cancer cells and review drugs that target metabolic pathways. We then discuss the effect of cancer and cancer therapy on systemic metabolism. Finally, the role of diet and lifestyle factors in carcinogenesis and response to therapy is reviewed.

Cancer Cell Metabolism

One of the earliest and most recognized metabolic alterations in cancer cells is increased glucose consumption by tumors. Elevated glucose uptake by tumors is detected by fluorodeoxyglucose-positron emission tomography (FDG-PET) imaging for initial cancer staging, assessing response to therapy, and surveillance. Beginning with the initial observation by Otto Warburg and others nearly a century ago that tumor cells increase glucose uptake and produce high quantities of lactate, even in the presence of oxygen, it has been well established that cancer cells engage in altered metabolism.[8-11] Through much of the genomic era, from the inception of gene cloning technologies and subsequent cancer gene discovery, cancer biology was focused on how signaling pathways and transcription factors control cancer growth and the cell cycle. However, in recent years, there has been a renewed interest in understanding how altered metabolism contributes to cancer pathogenesis. Many factors, such as tumor hypoxia, stromal composition, immune cell infiltration, and genetic alterations, play critical roles in defining cancer cell metabolism. Genetic and/or epigenetic

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alterations can provide a crucial survival advantage to cancer cells in a nutrient-starved environment. The renewed interest in cancer cell metabolism came initially from recognizing that dysregulated signaling pathways and transcriptional reprograming result in metabolic alterations in cancer cells.[12-15] Subsequently, it has been shown that the induction of oncogenes and/or loss of tumor suppressors is/are sufficient to drive metabolic changes in cancer cells.[16-19] Indeed, tumor-associated metabolic alterations are recognized as an emerging cancer hallmark.[1,20] In this section, we briefly review select examples of how metabolism is altered in cancer cells to highlight the diversity of mechanisms underlying rewired metabolism in cancer cells. For further information, the reader is referred to several excellent reviews of cancer metabolism.[12,13,15,21-24]

Glucose is the single most abundant nutrient for most cells and can be a source of biomass and fuel for energy production. Numerous signaling pathways altered in cancer affect glucose metabolism through a variety of mechanisms. In one classic paradigm, receptor tyrosine kinases induced by insulin or other growth factors activate the PI3K-AKT signaling pathway to stimulate glycolysis.[25] AKT (also known as protein kinase B) is a serine-threonine kinase that can increase glycolytic activity directly by phosphorylating hexokinase (the enzyme that catalyzes the first step in glycolysis) and indirectly by phosphorylation of substrates that regulate trafficking of glucose transporters 1 and 4 (GLUT1 and GLUT4) to the plasma membrane.[26-28] PI3K-AKT signaling also induces mammalian target of rapamycin complex 1 (mTORC1), which results in increased expression of hypoxia-inducible factor 1α (*HIF1α*).[29-32] HIFs are heterodimeric transcription factors consisting of an α-subunit (HIF1α and HIF2α), which is degraded in the presence of oxygen, and a stable β-subunit (HIF1β) (also known as ARNT).[33] In the presence of hypoxia, HIFs are stabilized and activate a transcriptional response that allows adaptation to hypoxic stress, including increased expression of *GLUT1* and *GLUT3*, hexokinase 2 (*HK2*), and some isoforms of phosphofructokinase 2.[34] HIF1α also promotes pyruvate dehydrogenase kinase expression, which inhibits pyruvate oxidation and shunts glucose metabolism toward lactate as an adaptation to hypoxic conditions.[35] HIF2α regulates other aspects of anabolic metabolism and is a therapeutic target in clear cell renal cell carcinoma.[36,37] Growth factor receptor signaling also activates RAS proteins to promote glucose uptake, glycolysis, and the pentose phosphate pathway. For example, in pancreatic ductal adenocarcinoma (PDAC), oncogenic KRAS drives glycolytic intermediates into the nonoxidative pentose phosphate pathway to support nucleotide production.[38]

Energy is maximally released when glucose is completely oxidized to CO_2, a process that produces ATP through oxidative phosphorylation (OXPHOS). When oxygen is limiting in cells, glycolysis becomes uncoupled from OXPHOS, and the end product of glycolysis, pyruvate, is instead reduced to lactate. Interestingly, in many cancer cells, glucose is preferentially catabolized through fermentation to lactate even when oxygen is not limiting (the *Warburg effect*). The conversion of pyruvate to lactate appears to be an essential mechanism by which cancer cells maintain an appropriate balance of redox cofactors to support biosynthetic functions.[2,39] It has been shown that loss of TP53 activity can push cells toward the glycolytic pathway instead of OXPHOS.[40] As first demonstrated nearly a century ago, tumors net dispose of lactate through systemic circulation, from where it is taken up by the liver and re-converted to glucose through gluconeogenesis (the *Cori cycle*).[41,42]. Recently, it has been reported that lactate may also be taken up by some cells in tumors to fuel the tricarboxylic acid (TCA) cycle,[43-45] but how this relates to increased glucose uptake remains an open question.[46]

In addition to glucose, increased uptake of other nutrients by cancer cells has been shown to support cancer cell survival, growth, and invasion. Oncogenic MYC has been linked to increased glutaminolysis, which results in glutamine addiction in some cancer cells.[47] Glutamine, like glucose, can provide carbons for lipogenesis, and MYC has been shown to regulate fatty acid metabolism.[48,49] Inactivation of the tumor suppressor retinoblastoma protein has also been shown to increase glutamine utilization because of the upregulation of glutamine transporter ASCT2 in human cancer cells.[50] Although glucose and glutamine are the most abundant nutrients in plasma and tissue culture media, the list of nutrients consumed by cultured cancer cells and tumors is vast. It is likely that, in most cases, the requirement for specific nutrients depends on tumor type and nutrient environment. For example, dependence on glutamine can be driven by high levels of extracellular cystine,[51] and some tumors are less dependent on glutamine metabolism in vivo.[52,53] As another example, many prostate cancers are not FDG-avid, yet they demonstrate increased uptake of [^{18}F]-fluciclovine (a synthetic analog of leucine) and [^{11}C]-choline used in PET imaging to detect metastases that are otherwise occult by conventional imaging.[54,55] How tumors balance glucose and glutamine metabolism with other nutrients available in their environment is an area of active investigation.

As demonstrated by the following examples, the cancer genomic era also brought the recognition that some metabolic enzyme are frequently mutated or amplified across a variety of cancer cell types. A recently published study analyzed a database from The Cancer Genome Atlas of >10,000 tumors across 32 cancer types and found at least one metabolic gene alteration per tumor with a varied number of metabolic gene alterations among cancer types.[56]

Germline mutations in 2 TCA cycle enzymes, fumarate hydratase and succinate dehydrogenase, predispose to cancer syndromes hereditary leiomyomatosis and renal cell cancer and hereditary paraganglioma-pheochromocytoma, respectively.[57,58] Affected individuals show loss of heterozygosity (or mutation) of the wild-type fumarate hydratase and succinate dehydrogenase allele in tumor tissue, which leads to the accumulation of fumarate and succinate, respectively. These metabolites, when present at high concentrations, inhibit DNA, histone demethylases, and prolyl hydroxylases, resulting in DNA hypermethylation, chromatin modification, and stabilization of HIF1α and HIF2α and other oncogenic factors.[59-61] Therefore, mutations in a single metabolic gene can result in altered metabolite levels, leading to transcriptional changes in cancer cells that promote uncontrolled growth.

Another prominent example of a metabolic enzyme that is frequently mutated in cancer is isocitrate dehydrogenase (IDH). Mutations in the *IDH-1* and *IDH-2* genes occur in a significant percentage of patients who have malignant glioma (60%-90%), chondrosarcoma (50%-70%), cholangiocarcinoma (10%-20%), acute myeloid leukemia (AML) (10%-20%), and, less frequently, in other cancers.[62] In glioma, *IDH* mutations are prognostic and predictive of response to the DNA alkylating agent, temozolomide.[63,64] Cancer-associated *IDH* mutations occur exclusively in the enzyme's active site, which creates *neomorphic* activity, resulting in excessive production of the *oncometabolite* D-2-hydroxyglutarate (D-2-HG).[65,66] When D-2-HG levels build up in tumors, it blocks the activity of 2-oxoglutarate–dependent DNA and histone demethylases. This, in turn, leads to hypermethylation and silencing of genes, including the enzyme O^6-methylguanine-DNA methyltransferase, which reverses DNA damage caused by temozolomide.[67-70] Therefore, a metabolite produced at high levels exclusively in IDH-mutant gliomas increases these cancers' sensitivity to temozolomide.

As pointed out in the examples above, mutations or deletions in metabolic enzymes can cause an abnormal accumulation of intracellular metabolites that lead to altered protein function through allosteric regulation. In addition, genes encoding metabolic enzymes can also undergo amplification through copy number alteration. For example, amplification of phosphoglycerate dehydrogenase (*PHGDH*), the first rate-limiting enzyme in the serine synthesis pathway, is observed in various cancer types, including a subset of melanomas and triple-negative breast cancers.[71,72] Serine is a proteinogenic amino acid that is also a significant source of one-carbon units for the folate cycle, which is required for de novo synthesis of purines and thymidine and, in some settings, has also been shown to contribute to NADPH production.[73] Some cancer cells require *PHGDH* amplification to obtain sufficient serine to support cancer cell proliferation.[71,72]

Cell lineage is also recognized as an important determinant of cancer cell phenotypes, including metabolic activity, proliferative index, metastatic potential, and response to therapy. As organs and tissues are formed during development, genetic programs are executed that determine cell fate. These developmental programs ultimately drive progenitor cells to become specialized cell types with unique metabolic activities designed to support the function of a given tissue type. During carcinogenesis, metabolic activity is rewired to support cancer growth, yet how cancer and associated stromal cells adapt their metabolism depends on the tissue and cell of origin of the cancer.[74-76] Cancer cells can maintain specific metabolic signatures, analogous to how cancer cells maintain lineage markers that reflect the tissue/cell type of origin.[77] For example, it has been described that primary prostate cancer and prostate cancer metastases maintain increased production of citrate, a metabolite that is secreted in high quantities by normal prostate glands.[78]

In addition to metabolic alterations that are intrinsic to cancer cells, it is also increasingly recognized that metabolic alterations occur in other cells within the tumor microenvironment; these changes can also contribute to cancer progression (reviewed by Elia and Haigis[79] and Lyssiotis and Kimmelman[80]). Furthermore, cancer cell heterogeneity and plasticity can also manifest as metabolic heterogeneity. Several studies have attempted to characterize metabolic heterogeneity in cancers.[81-83] However, this remains a challenging area of research given the inherent limitations of assessing metabolism at the single-cell level.

Immuno-oncology is an active area of research that has increasing intersection with the field of metabolism.[84,85] Significant energy goes into the production of cytokines, chemokines, and immune mediators, immune cell activation, and expansion, suggesting that changes in energy availability can affect the immune response.[86,87] Clinical studies have shown a clear connection between autoimmune diseases, such as rheumatoid arthritis and systemic lupus erythematosus, and immune cell metabolism.[88,89] Immune cells rely on specific metabolic pathways for activation, and manipulating these metabolic dependencies could present a unique opportunity to target diseases. For instance, activation of the glycolytic pathway in CD4 T cells promotes an inflammatory phenotype, whereas increased fatty acid oxidation skews them toward a regulatory phenotype.[90] B-cell and T-cell receptors directly activate transcription factors, such as c-MYC, HIF1α, PI3K, mTOR, and FOXO1, which play a critical role in immune cell metabolism and subsequent immune response.[91-93] Similarly, M1 macrophages rely on glycolysis and glutaminolysis pathways, whereas M2 macrophages prefer the oxidative phosphorylation pathway to meet the high demand for energy production during the activation phase.[94] Of clinical interest, it has been shown that blocking glutamine metabolism can enhance antitumor immune responses.[95]

In summary, there are multiple and often complex interactions between metabolic pathways and signaling pathways that, together, result in metabolic reprogramming, a fundamental hallmark of cancer. Furthermore, both intrinsic factors (genomic/epigenomic alterations) and extrinsic factors (nutrients, drugs, hormones, and interactions with stromal cells, extracellular matrix, and the immune system) contribute to the metabolic reprogramming of cancer cells. For further information, the reader is referred to recent reviews of these topics.[2,79,96]

Cancer Therapies That Target Metabolism

Although multiple mechanisms can contribute to metabolic reprograming in cancer, regardless of the underlying mechanism(s), the altered activity of metabolic enzymes provides an opportunity for therapeutic intervention if such activity is required for tumor maintenance (often referred to as a *metabolic dependency*) and if inhibition of the activity can be tolerated by host metabolism. In this section we provide select examples of the major classes of cancer drugs that target metabolic enzymes (Table 1).[97-143] For a more comprehensive discussion, the reader is referred to other excellent reviews of this topic.[4,144-146]

From the earliest days of cancer research, the increased proliferative index of cancer cells was recognized as a metabolic vulnerability. The observation that rapidly proliferating cells can be killed by agents that interfere with DNA replication led to the development of the first chemotherapeutic agents, including small molecules with direct genotoxic effects, such as the nitrogen mustards and drugs targeting nucleotide synthesis.[147,148] Because of their structural similarity to native metabolites, this latter class, termed *antimetabolites*, saw early successes that led to the rapid expansion of drugs targeting enzymes involved in nucleotide metabolism. One of the earliest drugs to treat cancer was methotrexate, an antifolate drug that inhibits thymidine synthesis.[149] Similarly, the related folate analog, aminopterin, inhibits one-carbon metabolism necessary for de novo nucleotide synthesis and was found to be effective in children with acute lymphoblastic leukemia.[150] Early clinical successes with these agents paved the way for the rapid development of additional small molecule inhibitors of nucleotide synthesis enzymes, many of which continue to form the backbone of multiagent chemotherapy regimens, such as inhibitors of dihydrofolate reductase and other folate-using enzymes, thymidylate synthase, phosphoribosyl pyrophosphate amidotransferase, ribonucleotide reductase, and other enzymes involved in purine and pyrimidine synthesis and salvage (Table 1) (reviewed in Scott, 1970[151]; Chaber and Roberts, 2005[152]; and Parker, 2009[153]).

Apart from targeting nucleotide and DNA synthesis, early cancer treatments have also targeted other metabolic pathways. For instance, it was discovered that acute lymphoblastic leukemia cells rely on exogenous asparagine for growth,[154] which led to the use of the bacterial enzyme L-asparaginase to limit the availability of asparagine for leukemia cell growth.[130] More recently, efforts have focused on developing agents to deplete other amino acids and to target central metabolic pathways aberrantly regulated in cancer cells, including glycolysis, the TCA cycle, and lipogenesis. Many of these agents are still in preclinical stages; however, some are now undergoing evaluation in clinical trials (Table 1).

Targeting central metabolic pathways always raises questions about therapeutic window because the alteration of systemic metabolism can have harmful effects. For example, targeting glycolysis directly has proven to be challenging because of the low therapeutic index of glucose uptake inhibitors such as 2-deoxyglucose, which affects glucose uptake in both cancer and normal cells.[114,155] However, the recognition that cancer cells have altered regulation of central metabolic pathways led to renewed interest in this field.[156] An example of this is pyruvate kinase (PK), which catalyzes the last step in the glycolytic pathway. Different tissues express different isoforms of PK.[157] Most cancer cells express a PK isoform (PKM2) that is different from the one expressed in erythrocytes (PKR), liver (PKL), myocytes (PKM1), and brain (PKM1). Multiple mechanisms have been proposed for why PKM2 is advantageous to cancer cells.[158] Preclinical studies have shown that drugs targeting PKM2 can be effective in some cancer types, and, in early trials of those drugs for other indications, they have proven safe in patients.[115,159-161] Other drugs targeting glucose metabolism, such as GLUT1 inhibitors (WZB117 and BAY876), GLUT4 inhibitors (silibinin and ritonavir), and a GLUT2 inhibitor (quercetin), are in various stages of clinical development.[112,144,162] In cancer cells, a significant proportion of glucose is metabolized to lactate and secreted by monocarboxylate transporters present on the plasma membrane. Inhibitors of lactate dehydrogenase A, such as quinoline, 3-sulfonamides, FX11, and PSTMB, are being investigated in preclinical settings.[116,163] An monocarboxylate transporter 1 inhibitor, AZD3965, is in early phase clinical trials for patients with solid tumors, diffuse large B-cell lymphoma, and Burkitt lymphoma.[119] GAPDH is another target with a therapeutic window determined by the extent of the Warburg effect on glycolysis.[164,165]

Amino acids are also important nutrients that support anabolic metabolism in cancer cells. Glutamine is the most abundant amino acid in the blood, and glutamine dependence has been observed in many cancer cell lines.[166] Although glutamine is a nonessential amino acid, it is an

TABLE 1. **Cancer Therapies/Drugs That Target Metabolic Pathways**

METABOLIC PATHWAY	TARGET[a]	DRUG	STAGE OF DEVELOPMENT	REFERENCES
Nucleic acid synthesis	Thymidylate synthase (TS)	5-Fluorouracil, capecitabine, pemetrexed, raltitrexed	Approved	Heidelberger 1957,[97] Jackman 1991,[98] Chin 1997,[99] Miwa 1998[100]
	Dihydrofolate reductase (DHFR)	Methotrexate, pemetrexed	Approved	Chin 1997,[99] Myer 1950,[101] Wright 1951[102]
	Glycinamide ribonucleotide formyltransferase (GARFT)	Pemetrexed	Approved	Chin 1997[99]
	Dihydroorotate dehydrogenase (DHODH)	Brequinar, leflunomide	Phase 1/2	Sykes 2018[103]
	Ribonucleotide reductase (RNR)	Gemcitabine, clofarabine, fludarabine, cladribine, cytarabine	Approved	Xie & Plunkett 1996,[104] Heinemann 1990,[105] Greene 2020,[106] Evans 1961,[107] Hertel 1990[108]
	5-Phosphoribosyl-1-pyro-phosphatase (PRPP) amidotransferase	Mercaptopurine, thioguanine	Approved	Skipper 1954,[109] Atkinson & Murray 1965,[110] Hill & Bennett 1969[111]
Glycolysis	GLUT1	WZB117, BAY-876	Preclinical	Ma 2018,[112] Liu 2012[113]
	Hexokinase	2-Deoxyglucose	Phase 1/2	Dwarakanath 2009[114]
	Pyruvate Kinase M2 (PKM2)	TEPP-46	Preclinical	Anastasiou 2012[115]
	Lactate dehydrogenase A (LDHA)	Quinoline, 3-sulfonamides, FX11, PSTMB	Preclinical	Kim 2019,[116] Billiard 2013,[117] Le 2010[118]
	Monocarboxylate transporter 1 (MCT1)	AZD3965	Phase 1	Marchiq & Pouyssegur 2016[119]
Glutamine metabolism	Glutaminase 1 (GLS1)	CB-839, IPN60090	Phase 1/2	Xiang 2015,[120] Gross 2014,[121] Soth 2020[122]
	ASCT2 (SLC1A5)	GPNA	Preclinical	Yoo 2020,[123] Esslinger 2005[124]
	Multiple targets	JHU-083	Preclinical	Leone 2019,[95] Hanaford 2019[125]
Amino acid transport and biosynthesis	Phosphoglycerate dehydrogenase (PHGDH)	CBR-5884, NCT-503	Preclinical	Wang 2017,[126] Pacold 2016,[127] Mullarky 2016[128]
	Indoleamine-2,3-dioxygenase-1 (IDO1)	Epacadostat, indoximod	Phase 3	Prendergast 2017[129]
	Circulating asparagine	L-Asparaginase	Approved	Clavell 1986[130]
	Large neutral amino acid transporter (LAT1)	JPH203	Preclinical	Enomoto 2019,[131] Oda 2010[132]
Mitochondrial metabolism	Pyruvate dehydrogenase (PDH), α-ketoglutarate dehydrogenase	CPI-613	Phase 2	Zachar 2011[133]
	Electron transport chain complex 1	Metformin, IACS-010759	Phase 1-3	Molina 2018,[134] Yam 2019[135]
Lipid metabolism	ATP-citrate lyase (ACLY)	SB-204990	Preclinical	Hatzivassiliou 2005,[136] Shah 2016[137]
	Acetyl-CoA carboxylase (ACC)	Soraphen-A	Preclinical	Svensson 2016,[138] Corominas 2014[139]
	fatty acid synthase (FASN)	TVB-2640	Phase 2	Mullen & Yet 2015[140]
Enzymes mutated in cancer	Mutant isocitrate dehydrogenase 1 (IDH1)	AG-120, BAY1436032, LY3410738, FT-2102	Phase 1-3	DiNardo 2018,[141] Heuser 2020[142]
	Mutant isocitrate dehydrogenase 2 (IDH2)	AG-221	Phase 3	Stein 2017[143]

[a]Key targets of nucleoside analogs are shown; however, most nucleoside analogs inhibit multiple nucleic acid and DNA synthesis/repair enzymes, including DNA polymerase.

important nitrogen donor for the biosynthesis of diverse compounds, such as glutathione, hexosamine, nucleotides, fatty acids, and nonessential amino acids. Furthermore, glutamine carbon can feed directly into the TCA cycle. In fact, a glutamine antagonist, JHU083, produced a potent antitumor response in combination with immune checkpoint blockade therapy in an animal model.[95] One route of glutamine catabolism to supply carbon to cells involves the enzyme glutaminase. Drugs targeting glutaminase, such as CB-839 and IPN60090, have been effective in some preclinical models and are now in trials for various malignancies.[120-122,167] Interestingly, targeting glutaminase appears to cooperate in preclinical settings with immune therapies, including chimeric antigen receptor T-cell therapies.[168] In addition, for several glutamine transporters, such as SLC1A5, LAT1, and SLC6A14, inhibition showed promising results in preclinical settings and are being actively pursued for future clinical applications.[123,131] Serine is another proteinogenic amino acid essential for nucleotide biosynthesis via its role as a donor of one-carbon units for the folate cycle.[169] As previously discussed, PHGDH, the enzyme that catalyzes the first step in the serine biosynthesis pathway, is amplified in some cancers, and, in this context is essential for proliferation.[71,72] Small molecule drugs targeting PHGDH are effective in some preclinical models, including inhibition of brain metastases.[126-128,170] Indoleamine-2,3-dioxygenase-1 (IDO1) is the critical enzyme in tryptophan catabolism, and elevated levels of IDO1 have been associated with poor outcomes in patients with cervical cancer and glioblastoma multiforme.[171,172] The inhibitors of IDO1 (epacadostat and indoximod) are in clinical trials in combination with other anticancer agents.[129]

Compared with most normal tissues, cancer cells have an increased demand for fatty acids to generate lipid membranes and precursors for signaling molecules. Fatty acids can be acquired exogenously through diet or synthesized endogenously from glucose, glutamine, or acetate.[173] Lipogenic enzymes are upregulated in many cancer cells, and de novo fatty acid synthesis has been viewed as a potential therapeutic target in cancer cells.[174,175] Three lipogenic enzymes have been a particular focus of drug development efforts, including ATP-citrate lyase, acetyl-CoA carboxylase, and fatty acid synthase.[136-140] The success of these agents in the clinic will likely depend on identifying tumors that are unable to take up sufficient lipids from their environment and thus require increased de novo fatty acid synthesis for survival. Although aberrant fatty acid metabolism helps cancer cells satisfy higher energy demand, it is also associated with increased lipotoxicity. Cancer cells need to maintain a proper ratio of saturated to unsaturated fatty acids to avoid mitochondrial dysfunction, excess reactive oxygen species, and endoplasmic reticulum stress.[176,177] To overcome lipotoxicity, cancer cells overexpress different isoforms of stearoyl-CoA desaturases (SCDs), which convert saturated to monounsaturated fatty acids. SCD1 is overexpressed in various cancer types, and SCD1 inhibitors are under investigation in preclinical studies.[178-182]

Although most drugs that target metabolism do not discriminate between cancer and normal cells, in the case of IDH-mutant cancers, there is a unique opportunity to target cancer cells selectively. Indeed, several drugs have been developed that selectively target the mutated enzyme.[183-185] This strategy has been effective in treating IDH-mutated AML.[141-143] IDH inhibitors are also in clinical trials for IDH-mutated solid tumors (glioma, chondrosarcoma, and intrahepatic cholangiocarcinoma); however, preclinical studies have suggested that IDH inhibitors may be less effective in solid tumors, and it has been suggested that this may be because mutant IDH activity is a driver of cancer initiation by altering epigenetic signatures, but it may be less important in tumor maintenance at later stages of tumor progression.[4,186-188] It is worth pointing out that, across many solid tumor types, drugs that target metabolic enzymes are effective in slowing tumor growth in preclinical models, yet tumor regression is rarely observed, indicating that some of these agents may be most useful as part of multiagent regimens or as maintenance therapy.

Systemic Metabolic Effects of Cancer

Weight loss is a common presenting symptom in patients with cancer, and more than one-half of patients who have cancer experience anorexia at baseline, the etiology of which remains poorly understood.[189-191] Cancer cachexia is a severe form of wasting characterized by loss of lean body mass or sarcopenia with or without loss of fat mass.[192] This contrasts with starvation, in which liver mass and fat mass are lost while lean body mass is initially preserved. Numerous circulating factors, including cytokines, neuropeptides, eicosanoids, and tumor-derived proteins, have been implicated in the pathogenesis of cachexia, and the underlying etiology is likely multifactorial.[193-195] Increased resting energy expenditure has been observed in some patients with cancer, and its prevalence may depend on cancer type, indicating that hypermetabolism may be a feature of some cancers that contributes to the wasting phenotype.[196,197]

Although anorexia likely contributes to cancer-associated weight loss and cachexia, hyperalimentation with parenteral nutrition does not improve treatment outcomes and only partially reverses the wasting phenotype, indicating that the pathophysiology of cachexia is more complex than simply reduced calorie intake secondary to anorexia.[198,199] In a randomized clinical trial of patients with small cell lung cancer, parenteral nutrition temporarily improved body fat yet had no effect on lean body mass or survival.[200] Cachexia is an early presenting sign in many patients with PDAC, and decreased exocrine function has been implicated as a contributory factor.[201-203] Yet, surprisingly, despite reversing some tissue wasting, replacement of pancreatic enzymes did not improve survival in a murine PDAC model.[202] Furthermore, in patients with PDAC, cachexia is not necessarily associated with worse survival.[202] Nevertheless, pretreatment weight loss is a poor prognostic factor in several cancers,[191] and the systemic metabolic effects of cancer are heterogeneous, with much left to understand.

Cancer cachexia and weight loss are also associated with systemic metabolic changes, including derangements in glucose, lipid, and protein metabolism.[204-207] These systemic metabolic alterations have been attributed to changes in host metabolism induced by the tumor rather than the metabolic activity of the tumor itself.[208] This contrasts with animal models in which the tumor-to-host mass ratio is often large, and tumor metabolism can cause direct systemic metabolic changes.[209] For the most part, early human studies failed to show significant metabolic changes in patients with early stage localized cancer, leading to the assumption that the metabolic changes were not because of cancer per se but rather a manifestation of a cancer-associated wasting phenotype.[208] However, as discussed in further detail below, technological advances resulting in improved metabolite detection and resolution have led to the discovery of distinct systemic metabolic signatures in patients with cancer, even at early stages and, in some cases, even before the disease becomes clinically apparent.[210]

Metabolic Effects of Cancer Therapy

In addition to the aforementioned cancer-induced systemic metabolic changes, treatment of cancer with surgery, radiation, systemic therapy, or hormonal therapy causes acute and long-term side effects that also can affect metabolism. Not surprisingly, side effects involving the digestive system account for the majority of acute treatment-related toxicities affecting metabolism.[211] Malnutrition and weight loss may result from nausea, vomiting, diarrhea, mucositis, and dysgeusia, which are common in patients receiving treatment for head and neck and gastrointestinal malignancies. Resting energy expenditure initially decreases in patients undergoing chemoradiotherapy for head and neck cancer and increases toward the end of treatment.[212] It has been suggested that the increased energy expenditure at the end of therapy is related to stress caused by the cumulative effects of chemoradiotherapy.[212] A systematic review of the literature on energy metabolism in patients who received chemotherapy for all cancer types showed no universal impact of chemotherapy on energy expenditure but suggested that patients receiving chemotherapy become hypometabolic during treatment.[213] Most studies have used indirect calorimetry, anthropometry, and routine laboratory tests to assess metabolic status. There is comparatively little metabolomic data on changes that occur during cancer therapy. A small study using stable isotope tracers showed no significant difference in glucose, lipid, or protein metabolism in patients undergoing radiotherapy for head and neck and lung cancer.[214] Whereas combined modality therapy (surgery, radiation, and chemotherapy) for soft tissue sarcoma had significant acute nutritional effects, patients who were disease-free after treatment showed minimal nutritional morbidity.[215]

Although acute metabolic side effects of aggressive cancer therapy can be pronounced, fortunately, long-term side effects tend to be more subtle. Incidence and severity depend on treatment modality and interval since treatment.[216] Other than long-term sequelae of decreased endocrine/exocrine function after surgery or radiation involving the pituitary, thyroid, adrenal, pancreas, and gonads, there are relatively sparse data on the long-term metabolic effects of these modalities.[217] Nevertheless, even low doses of total body radiation in the pediatric population significantly increase the risk of long-term endocrine disturbance and metabolic abnormalities.[218] In contrast to local therapies, systemic therapy carries a higher risk of long-term metabolic derangements. The long-term metabolic effects of hormonal therapy in patients with breast and prostate cancer are well documented, including chronic effects on mineral and lipid metabolism.[219-221] In addition, prior chemotherapy is associated with chronic weight gain in breast cancer survivors, with the highest risk in premenopausal women.[222] Long-term metabolic effects of therapy in other cancers and potential pathophysiologic mechanisms have been reviewed.[216] Unfortunately, much of the prior data are insufficient to establish causation. Broader use of metabolomic analysis in clinical trials and surveillance protocols could provide a better understanding of pathologic mechanisms and identify opportunities for intervention.

The Role of Diet and Lifestyle Factors in Carcinogenesis and Response to Treatment

Metabolism has also been the focus of research aimed at identifying risk factors for cancer. Epidemiologic studies demonstrate a positive correlation between cancer incidence and deleterious metabolic states, including obesity and diabetes.[223-225] In addition, dietary factors and physical inactivity have been implicated.[226-228] Furthermore, in the postdiagnosis setting, the relevance of these factors to disease progression, recurrence risk, and mortality has been demonstrated for some cancers.[229-236] Collectively, these data have led to the adoption of guidelines on healthy food choices and physical activity to reduce cancer risk.[237]

Conceptually, dietary composition can affect circulating nutrients and metabolic hormones, which may directly affect metabolism within tumor cells.[238,239] Whereas the effect of diet, exercise, and systemic metabolic status on cancer initiation can be challenging to model experimentally, the impact of diet on tumor progression has been extensively evaluated in mouse models of various cancers, including breast, prostate, lung, pancreas, liver, intestine, and others.[240,241] In line with human data, laboratory studies in rodents generally support the notion that, in some cases, caloric restriction and ketogenic diet can slow tumor growth, whereas a high-fat diet can promote tumor progression.[242-246] However, as some studies show, this is a gross oversimplification, and the effect of diet on tumor progression likely also depends on tumor genetics and the tumor microenvironment.[247,248] Furthermore, mechanistically, it has been difficult to ascertain the extent to which individual nutrients directly affect tumor growth instead of indirect effects on growth factor signaling. For example, insulin-like growth factor (IGF) signaling has been implicated in many caloric restriction studies, yet direct activation of IGF only partially reverses the effect of caloric restriction on tumor growth.[249-251] Because manipulating one dietary factor or nutrient can affect many others, future studies will need to address the effects of diet and exercise on the metabolomic landscape both systemically and ideally within the tumor microenvironment.

In addition to impacting carcinogenesis, diet and lifestyle factors may also affect cancer therapy.[252-254] A low-calorie, low-protein diet improved objective tumor response in patients receiving neoadjuvant chemotherapy for breast cancer.[255] In animal models, caloric restriction and other dietary perturbations have been shown to alter the efficacy of chemotherapy and radiotherapy via metabolite-specific effects on IGF signaling, oxidative stress, and nucleotide metabolism.[256-258] Although data from human observational studies and mechanistic studies in laboratory animals are compelling, results from human interventional trials, for the most part, have been disappointing and highlight the limitations of a reductionist approach. It has been suggested that a more unified approach combining genomics, metabolomics, and biomarkers may be more effective at identifying modifiable risk factors in the context of heterogeneous dietary patterns and tumor characteristics.[259] A conceptual framework for investigating dietary effects on tumor metabolism has been proposed.[260] The key elements build on clinical and laboratory observations that dietary manipulation can alter nutrients in the tumor environment, and this, in turn, can impact tumor metabolism, growth, progression, and response to therapy. Indeed, depletion of specific amino acids can slow growth of some tumors,[256,261-265] and, if we extend this framework to account for complex interactions between diet, exercise, stress, inflammation, and the microbiome on micronutrients and growth-activating/growth-inhibitory signals in the tumor environment, it is clear that a multiomics approach is needed to identify actionable diet and lifestyle factors that can impact response to cancer therapy.

Defining Metabolomics

Historically, human disease has been studied using a reductionist approach to separate and identify individual factors that cause or contribute to a pathologic state. In contrast, systems biology seeks to understand complex biological systems by considering the sum of its molecular constituents and how they interact to define a phenotype.[266] At the heart of systems biology is an ability to measure many aspects of cell state, which has been facilitated by advances in genomics, transcriptomics, proteomics, and metabolomics.

In concept, metabolomics stands apart from the other -*omics*, in that it provides a functional readout of metabolic processes and thus is a direct assessment of phenotype (Fig. 2). That is to say, metabolomics, if interpreted appropriately, can provide a readout of the sum of alterations occurring at the DNA, RNA, and protein levels and, in some cases, may be the most sensitive way to identify pathologic variants because even small changes in protein expression or structure can lead to significant changes in protein activity and metabolite levels.[267] Conversely, metabolites can alter protein activity and thereby affect nearly every biological process, including DNA replication, RNA transcription, and translation (Fig. 2). The term *epimetabolites* has been used to describe a subset of metabolites that function as active biomarkers and are involved in diverse biological functions, including epigenetic regulation, tumorigenesis, cancer cell invasion, cancer stem cell pluripotency, insulin sensitivity, and other cellular processes.[268] Furthermore, metabolomics also considers alterations in the tumor environment, including therapeutic interventions that can exert selective pressures on tumor subclones and thus shape the genome, transcriptome, and proteome.[269] Understanding the metabolic milieu thus has important implications for interpreting genomic, transcriptomic, and proteomic data.

In practice, metabolomics is defined as the analysis of small molecule metabolites (≤1500 Daltons and nonpeptide) in a biological specimen.[270] It involves the simultaneous identification of hundreds to thousands of chemicals based in part on the chemical properties and/or weight of atoms within a molecule (described further in the section below). This contrasts with standard clinical measurement of metabolites, such as glucose and urea, which relies on identifying chemicals based on enzymatic reactions and requires a separate test for each metabolite. The field of metabolomics has benefitted greatly from relatively recent technological advances in instrumentation, resulting in more affordable instruments with a smaller footprint. In addition, vendors have focused on delivering more user-friendly acquisition software and computational analysis tools. Validated data sets have made it easier to quickly and accurately process data. In addition there are many open-source software packages available for analyzing metabolomics data.[271] Nevertheless,

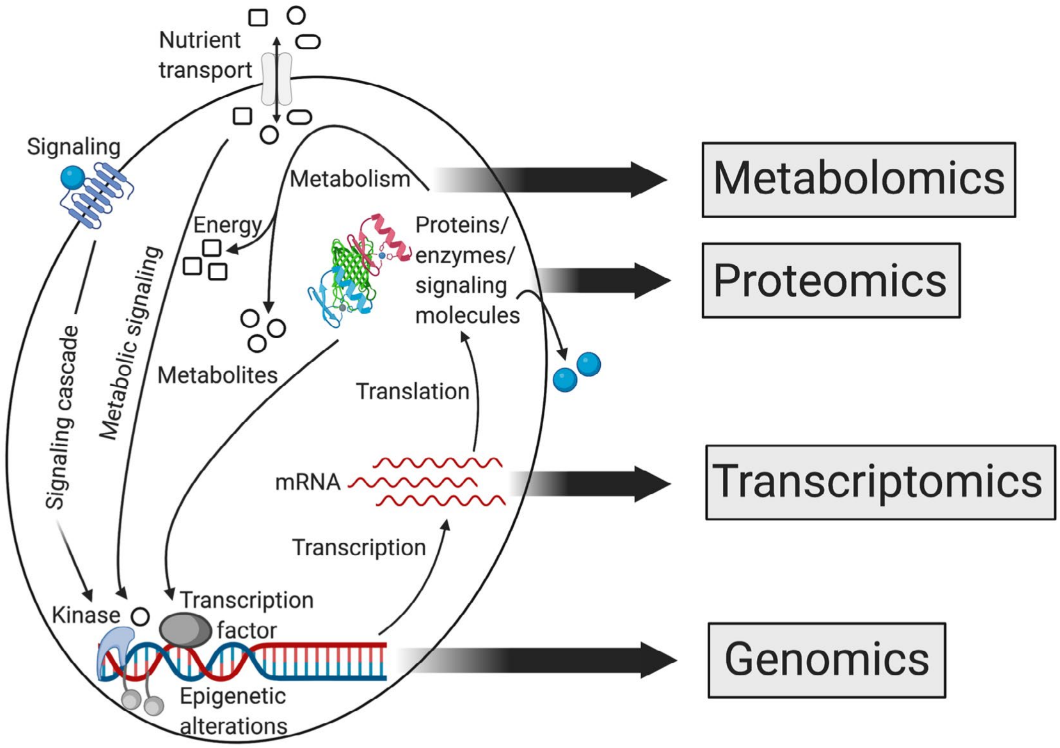

FIGURE 2. The relationship between *-omics* approaches of systems biology. Cancer is caused by changes at the genomic level that result in altered RNA transcription, protein expression, and protein function. The metabolome provides a functional readout of these upstream changes. In turn, individual metabolites affect protein activity and thereby alter RNA transcription and DNA replication.

appropriate experience and training of staff can still be a bottleneck for smaller academic/clinical laboratories.

As discussed below, multiple methods exist for metabolomics analysis, each with its own advantages and disadvantages; however, the first decision point in selecting the appropriate method is whether an untargeted or targeted approach is desired. Generally, untargeted metabolomics is used for hypothesis generation and is used extensively in biomarker discovery, whereas a targeted approach (that is, defining the metabolites that will be measured *before* performing the analysis) is used when testing a specific hypothesis or in validation and implementation stages.[272] In genomics, transcriptomics, and proteomics, the objective is to identify the macromolecular structure from a sequence of chemical constituents (nucleotides, amino acids) that are well defined and relatively limited in diversity. Because the chemical composition is constrained, a complete analysis is obtained by applying a single protocol. In contrast, metabolomics deals with a chemically diverse and complex set of molecules. Because lipids, sugars, organic acids, and other polar molecules have a wide range of physical characteristics, multiple methods for sample preparation and data acquisition are needed for their analysis. Furthermore, metabolites that are annotated by software in untargeted experiments require subsequent validation with a chemical standard using a targeted method. Consequently, analyzing differences between groups detected by untargeted metabolomics requires considerable expertise and effort, particularly if it is desirable to assign identities to each species measured. Therefore, the usefulness of untargeted metabolomics in aiding biological understanding and interpretation is limited by the ability to identify unknown metabolites.[272]

Methodology and Instrumentation

In this section, we introduce the major technologies that are used for metabolomics (Fig. 3). We focus primarily on methods involving mass spectrometry (MS) and nuclear magnetic resonance (NMR), which can both be used for compound detection in any biofluid or cell/tissue extract in the liquid phase. NMR can also be used with solid-phase samples, such as cell membranes or even intact cells and tissues. Other techniques involving analysis of metabolites in situ, such as matrix-assisted laser desorption/ionization mass spectrometric imaging (MALDI-MSI), or NMR-based in vivo imaging, such as magnetic resonance spectroscopic imaging (MRSI), are more limited in their application and have been reviewed elsewhere.[273,274]

NMR relies on measuring chemical properties of specific atoms in a molecule. NMR is a versatile method as it can be used with biospecimens in liquid, solid, or gas phase without prior processing. A sample is subjected to a strong magnetic field and then pulsed with radiofrequency waves. The energy

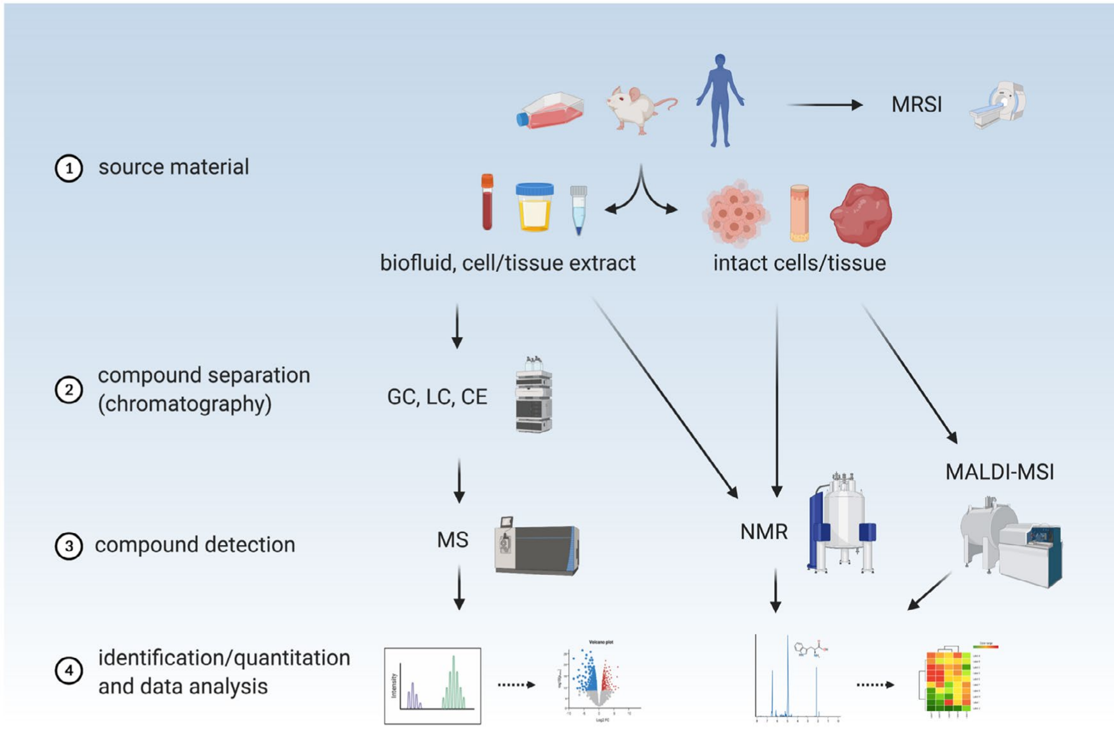

FIGURE 3. The optimal metabolomics workflow depends on source material and application. Various technologies and methods can be used to acquire raw data, which then provide the starting point for computational analysis. See text for details. CE indicates capillary electrophoresis; GC, gas chromatography; LC, liquid chromatography; MALDI-MSI, matrix-assisted laser desorption/ionization mass spectrometric imaging; MRSI, magnetic resonance spectral imaging; MS, mass spectrometry; NMR, nuclear magnetic resonance.

from radiofrequency radiation is used to transiently excite certain nuclei in a molecule (such as ^{1}H, ^{13}C, ^{15}N, or ^{31}P), causing them to flip their spin state when aligned in a strong magnetic field. As these nuclei relax, they produce a characteristic spectroscopic pattern (chemical shift) that reflects the type, location, and electromagnetic environment of excited atoms in a given molecule. Material is not consumed during NMR spectroscopy and thus can be used for subsequent analysis. The major drawback is the large footprint of NMR instruments and relatively low sensitivity (micromolar) compared with mass spectrometry (nanomolar).[275] Because most metabolites are relatively low-abundance in biological material, the diversity of chemical species that can be practically measured by NMR is also lower than for MS-based techniques. NMR is capable of resolving several hundred unique metabolites, whereas MS can be used to identify thousands of features with unique masses.

MS is the most widely used analytical technique in metabolomics. It relies on determining the ratio of mass to charge (*m/z*) of a molecule and/or its characteristic fragments. A sample in the liquid or gas phase is injected into the mass spectrometer, where metabolites become ionized and then separated by their *m/z*. Sample material is entirely consumed during this process. MS is typically coupled with an initial chromatographic stage, which increases the resolution of isobaric (same mass) compounds and improves the detection of less abundant species by reducing signal suppression by more abundant species. When coupled with gas chromatography (GC-MS), the technique is highly sensitive but generally requires initial chemical modification (derivatization) of metabolites to increase their volatility and make them suitable for separation in the gas phase. GC-MS can be used to analyze most polar, nonpolar, organic, and inorganic compounds; however, phosphorus-containing compounds (many sugars, lipids, and nucleotides) are challenging to detect using GC-MS because of difficulty generating volatile derivatives. In contrast to GC, liquid chromatography (LC) involves passing a liquid solution of metabolites over a solid-phase column, during which they are separated based on their chemical affinity for the solid phase. The chemical composition of the liquid phase is changed until all metabolites are eluted from the column. LC-MS does not typically require chemical modification of analytes and is well suited for the analysis of biofluids and phosphorus-containing compounds; however, it is necessary to use different solid-phase columns and different mobile-phase compositions for the optimal separation of compounds from different chemical classes. For example, lipidomics, which focuses on the lipid subset of the metabolome, involves chromatographic techniques that are fundamentally different from techniques used to analyze polar metabolites such as sugars or organic acids.[276] Nevertheless, given the combination of ease of use with biofluids, high sensitivity, and broad range of metabolites that can be measured, LC-MS is the most comprehensive and widely used analytical method associated with metabolomics. Capillary electrophoresis-MS is a related technique in which charged compounds are separated based on electrophoretic mobility (charge-to-size ratio) and thus can be useful to detect intrinsically charged metabolites (eg, choline) present in low-abundance and very small sample volumes.[277]

Although compound separation before injection into a mass spectrometer significantly increases sensitivity to detect low-abundance metabolites, the time needed for sufficient chromatographic separation of a complex biological sample can limit the number of samples that can be analyzed in a finite period of time. The addition of system suitability and quality-control samples also increases the run time for untargeted metabolomics experiments.[278] For example, a batch of 50 samples could take several days of instrument time. To increase throughput, biofluids can also be directly injected into a mass spectrometer without prior chromatographic separation. This technique, referred to as flow-injection MS, substantially increases throughput, allowing hundreds of samples to be run in a single day. The downside of flow-injection MS is that less abundant species can be missed; therefore, this technique is best suited for high-throughput, low-sensitivity analyses such as metabolic fingerprinting, in which the goal is to capture a snapshot of total metabolite content rather than identify individual metabolites. It can also be used as a screening tool for both untargeted metabolomics (when used together with optimized *m/z* scan ranges[279,280]), and -lipidomics (termed *shot-gun lipidomics*[281]). However, ultimately, these methods must be combined with traditional LC-MS approaches if identification of the detected features is required. This is especially important in lipidomics because shot-gun lipidomics cannot separate isobaric/isomeric lipid species, of which there are many.

Source Material

Metabolomics can be performed on a wide range of biological materials, including cells and tissues cultured in the laboratory, specimens collected from laboratory animals, and either freshly obtained or appropriately archived clinical specimens, including tumors and biofluids. The quantity of starting material depends on the technique used. For NMR, samples volumes are typically in the 0.1 to 0.5 mL range for liquid samples. MS-based methods are more sensitive, with 10-fold to 1000-fold lower limits of detection compared with NMR. Thus as little as a few microliters or milligrams of starting material can be sufficient to detect many metabolites.

In the laboratory, cells and tissues can be studied under controlled conditions in which levels of nutrients in the environment can be manipulated and isotope-labeled nutrients can be used to assess nutrient fate and determine flux through metabolic pathways.[282-284] Samples can be collected at predefined time points and rapidly processed. The acquisition, handling, and storage of clinical specimens are more complex and require careful planning and coordination between clinical and laboratory staff; however, with careful protocols, even stable isotope-labeled metabolite tracing can be accomplished in patients with cancer.[83,285] The most common types of clinical material that have been used for metabolomics analysis include blood, plasma, serum, urine, and extracts of biopsy or surgical specimens, examples of which are discussed in a section below. However, many other types of biofluids have also been used, including sputum, bronchial washings, saliva, sweat, tears, cerebrospinal fluid, pleural or ascitic effusions, fecal water, bile, breast milk, amniotic fluid, seminal plasma, expressed prostatic secretions, and others.[270,286-290] Regardless of the material used, the methods of collection, storage, and processing must be appropriately selected for the desired analysis. Many chemical species are labile or undergo rapid chemical modification by molecular oxygen or metabolic enzymes. Where applicable, appropriate quality controls are needed to ensure that chemical decomposition is minimized. Guidelines for reporting biospecimen source, collection, and processing have been set forth by the Metabolomics Society.[291]

Clinical Implementation of Metabolomics

Although both untargeted and targeted metabolomics have huge potential in biomarker discovery and hypothesis testing in the translational setting, several challenges still need to be overcome before metabolomics can become widespread in clinical research and practice. As described above, multiple complimentary methods are required to cover the entire metabolome. Often, this requires multiple instrumentation platforms, which may not be available in many academic and clinical laboratories. Furthermore, there are multiple software packages for data processing and analysis, particularly in untargeted metabolomics. Different peak-picking algorithms, for example, can yield slightly different results. Analyzing large metabolomics data sets also requires robust experimental design, which facilitates appropriate statistical analysis. Therefore, at a minimum, a successful metabolomics research study requires the combined efforts and expertise of analytical chemists, statisticians, and biologists. Furthermore, given the multiple methods and instrumentation platforms involved, as well as the various data processing algorithms, it is a logistical challenge to apply *industry standards* to the field in a way that is necessary in a clinical laboratory setting. Another major challenge for the clinical implementation of metabolomics is standardization of data analysis and reporting between institutions. Currently, most metabolomics studies yield relative quantification. In order for standardization to happen across platforms, absolute quantification would be required. Additional challenges and suggestions for new strategies have been reviewed extensively by Pinu et al.[292] In 2007, the Metabolomics Society launched a standards initiative to define the minimum reporting standards required for metabolomics data.[246] However, many published data sets still fall short of these minimum standards because of a lack of consensus across laboratories.

Clinical Applications

Metabolomics seeks to capitalize on the metabolic signature of cancer to assess disease risk or for earlier cancer detection, diagnosis of specific disease subsets, or treatment monitoring. Metabolomics in principle may also help inform the rational selection of targeted therapies to match the metabolic dependencies of cancer. In this final section, we discuss these clinical applications of metabolomics and provide select examples to illustrate how the metabolomics field has opened new opportunities in cancer research and is beginning to impact diagnosis and treatment.

Identifying Cancer Risk Factors

It is widely accepted that tumorigenesis involves sequential accumulation of genetic mutations that ultimately give rise to malignancy.[293] Although some oncogenic mutations and predisposing polymorphisms are germline-encoded, environmental factors promote the process of tumorigenesis by both inducing somatic DNA alterations and selecting for transformed cells. Small molecule carcinogens and ultraviolet or ionizing radiation cause DNA alterations by directly interacting with DNA. In contrast, inflammatory and metabolic factors contribute indirectly to tumorigenesis by inducing reactive oxygen species and creating an environment in which oncogenic mutations and epigenetic alterations are selected. In addition, altered metabolism affects the availability of substrates used to modify chromatin, thereby influencing chromatin dynamics and epigenetic changes that drive tumorigenesis.[294] Untargeted metabolomics on prediagnostic serum can uncover metabolic risk factors but requires access to material from large population-based cohort studies. Several examples are discussed in this section.

Early epidemiology studies focused on the contribution of one or a few metabolic factors on tumorigenesis. In the 1970s, multiple studies linked colorectal cancer to high-fat diet intake, low serum cholesterol, and high fecal bile acids.[295,296] Analysis of prediagnostic serum in prostate cancer cohort studies suggested a positive correlation between ω-6-polyunsaturated fatty acids and cancer risk, whereas ω-3-polyunsaturated fatty acids were inversely correlated with cancer risk.[297] Likewise, some polyunsaturated fatty acids, but not others, were linked to increased breast cancer risk.[298,299]

More recently, metabolomics has been used to examine the association between a broader range of metabolites in prediagnostic serum and cancer risk. Prospective analysis of circulating metabolites in patients with breast cancer revealed that acylcarnitine and phosphotidylcholines were strongly associated with the risk of breast cancer, regardless of breast cancer subtype, age, fasting status, menopausal status, or adiposity.[300] Similarly, higher circulating lysophosphatidylcholines were correlated with lower risks of breast, prostate, and colorectal cancer.[301] The examination of prediagnostic serum from the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial (ClinicalTrials.gov identifier NCT01696981) was the first to identify serum metabolites associated with coffee intake and found that some caffeine-related metabolites were inversely associated with colorectal cancer.[302] Another study examined metabolites in prediagnostic serum from postmenopausal patients with invasive breast cancer and matched controls from the PLCO trial and found that metabolites related to alcohol, vitamin E, and animal fats were associated with the risk of hormone receptor-positive breast cancer.[303] Metabolomic analysis of fatal prostate cancer cases and controls from the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study (ClinicalTrials.gov identifier NCT00342992) found that higher levels of amino acids involved in redox metabolism (thioproline, cystine, and cysteine) were associated with a reduced risk of lethal prostate cancer.[304] In contrast, leucylglycine and γ-glutamyl amino acids were associated with an increased risk of terminal prostate cancer, whereas 3-hydroxybutyrate, acyl carnitines, and dicarboxylic fatty acids were higher in patients who presented with de novo metastatic disease.[304] Other metabolomic studies using serum collected before cancer diagnosis in screening or prevention trials found associations between branched-chain amino acids and pancreatic cancer[210] and between pseudouridine and ovarian cancer.[305]

Despite these associations, a contentious issue has been whether implicated metabolites play a direct causal role in tumorigenesis or are merely an early manifestation of preclinical disease. For this reason, it has been suggested that incident cases occurring within 2 years of a nonlocalized cancer diagnosis be excluded from analyses searching for a direct causal role of metabolic factors in tumorigenesis.[306]

Identifying Biomarkers for Cancer Screening, Diagnosis, and Monitoring

Cancer detection techniques are essential not only for initial diagnosis but they also provide effective ways to screen appropriate populations, guide initial treatment strategy, assess treatment efficacy, and track cancer progression over time. In recent years, there have been several comprehensive reviews of metabolic biomarker studies in common cancers.[307–311] Here, we review some of the metabolite-based tests with established clinical applications and provide examples of metabolomic studies that are expanding the repertoire of metabolic biomarkers for cancer detection and surveillance.

Imaging techniques, including computed tomography, magnetic resonance imaging, PET, and radionuclide scans, are used extensively in the clinic for cancer detection and follow-up. One of the earliest metabolic markers used in cancer detection was FDG-PET.[312] It takes advantage of elevated glucose uptake by cancer cells, but, because glucose uptake also increases during inflammation, this limits the use of FDG-PET in some settings.[313] In addition to FDG, a wide range of radiolabeled carbohydrates, amino acids, and fatty acids have been used in preclinical and clinical settings to take the advantage of the high metabolic rate of tumors as a diagnostic tool. Other ^{18}F-labeled sugars, such as D-mannose, D-lactose, D-fructose, and D-galactose, have been developed and studied as PET tracers in preclinical settings and may allow the detection of some cancer cells that use a sugar molecule other than glucose for energy.[314-317] Some cancer cells overexpress specific amino acid transporters such that radiolabeled amino acids can be used to detect these cancers. For instance, amino acid utilization has been evaluated by [^{11}C]-tyrosine PET in soft tissue sarcomas and pituitary adenomas, whereas [^{11}C]-methionine PET has been evaluated in brain tumors.[318-320] To study the role of glutaminolysis in tumors, a series of glutamine-based PET tracers, including L-[5-^{11}C]-glutamine, [^{18}F]-(2S,4R)-4-fluoroglutamine, and [^{18}F]-(2S,4S)-4-(3-fluoropropyl)glutamine, have been synthesized.[321-323] A recent clinical study has shown high uptake of [^{18}F]-(2S,4R)-4-fluoroglutamine in patients with glioma, suggesting increased dependence on glutaminolysis.[324] Anti–1-amino-3-^{18}F-fluorocyclobutane-1-carboxylic acid, also known as [18F]-fluciclovine, is a synthetic analog of the amino acid leucine, the uptake of which is facilitated by amino acid transporters and is useful for the detection of recurrent prostate cancer.[54,325] Apart from amino acids and sugars, cancer cells also engage in elevated lipid metabolism to sustain rapid cell proliferation and survival. ^{11}C-fatty acids have been developed and used as PET radiotracers for studying β-oxidation in animals and humans.[326] Radiolabeled forms of the phospholipid head group choline, [^{18}F]-fluoromethylcholine and [^{18}F]-fluoroethylcholine, are used in the clinic for prostate cancer detection.[55,327]

In addition to PET imaging, MRSI is also emerging as a tool for noninvasive assessment of tumor metabolism.[328] For example, detection of D-2-HG by MR-spectroscopy in IDH-mutant glioma has been demonstrated in clinical studies and may have a role in monitoring patients receiving IDH-targeted therapy.[329,330] The use of MRSI in the clinic will likely expand as protocols become more standardized and efficient and as new metabolic biomarkers are identified.

Despite its established role in the clinic, there are several limitations to PET imaging, including limited availability and short half-life of some radiotracers, poor image resolution, inability to detect smaller tumors, and inability to distinguish tumors from nonmalignant hypermetabolic processes. For example, a meta-analysis of 45 different studies, which assessed lymph node involvement in patients with nonsmall cell lung cancer, concluded that the sensitivity and specificity of PET-computed tomography was roughly 75% and 90%, respectively.[331] Progress in understanding metabolic differences between tumor, normal tissue, and nonmalignant disease states will hopefully facilitate the implementation of tracers with greater diagnostic accuracy.

Compared with metabolic imaging, direct analysis of metabolites in clinical specimens and biofluids has the advantage of increased sensitivity and diversity of chemical species that can be monitored. When surgical specimens are available, metabolomic analysis of tumor and adjacent normal tissue can be used to identify metabolic pathways that are altered in cancer. For evaluating labile metabolites, rapidly collected and processed biopsy tissue is ideal; however, even formalin-fixed, paraffin-embedded archival tissue has been used for metabolic profiling.[332] In lung cancer, one of the most commonly elevated metabolites in tumor tissue and serum is lactate.[333-337] In fact, one study found that more aggressive lung cancers had higher lactate production, suggesting that lactate levels can be used to assess disease aggressiveness.[338] Similarly, glutamate is elevated in tumor and serum samples from patients with lung cancer.[333,335,337,339,340] Studies have shown that the elevated glutamate likely comes from a dependency of cancer cells for increased glutamine, which provides necessary nitrogen for the synthesis of nucleotides and amino acids.[341] Glutamate enrichment is also seen in breast cancer tissue compared with normal breast tissue.[342] In contrast, glutamine levels are lower in colon and stomach tumor tissue because of the high rate of glutaminolysis driving proliferation.[343] A wide range of other metabolites, including amino acids, purines, pyrimidines, and intermediates of metabolic pathways, have been found to be altered in cancer relative to normal tissue. For example, MS-based metabolomic analysis of human and mouse colorectal tumors identified 10 metabolites that were increased in tumor tissues compared with non-tumor tissues (proline, threonine, glutamic acid, arginine, N1-acetylspermidine, xanthine, uracil, betaine, symmetric dimethylarginine, and asymmetric-dimethylarginine); furthermore, these metabolites also showed detectable increases in urine of tumor-bearing mice.[344]

Metabolomic analysis of tumor tissue may also allow differentiation between tumor subtypes and aggressiveness of tumors.[335,345-347] By analyzing tumor tissue, plasma, and urine, sarcosine was identified to play a role in prostate cancer progression.[348,349] The role of sarcosine as a biomarker of prostate cancer in urine, however, has not been replicated in other studies.[350] In IDH-mutant AML, serum and urine D-2-HG levels have been evaluated as a tool to assess disease activity and therapeutic response.[351]

Although tissue biopsy is critical for establishing the initial diagnosis, it is neither practical nor desirable for cancer screening, monitoring, or surveillance. In contrast, biofluids, such as blood, serum, urine, saliva, and sweat, are a convenient source of material for biomarker detection. Serum antigen and hormone biomarkers exist for prostate adenocarcinoma, PDAC, ovarian cancer, nonseminomatous germ-cell tumors, thyroid cancers, hepatocellular carcinoma, and others. However, most of these biomarkers are not specific for cancer and thus must be interpreted together with other clinical or laboratory findings. There is intense interest in developing improved prognostic and diagnostic biomarkers based on molecular analysis of circulating tumor cells and cell-free DNA, RNA, or protein. In recent years, remarkable numbers of studies have also explored the use of biofluids as a source of metabolic biomarkers for cancer detection, treatment monitoring, and surveillance. For illustrative purposes, we discuss only a few examples and refer the reader to several reviews for more comprehensive coverage of this topic in different cancers.[310,352-354]

In breast cancer, 4 metabolites (L-octanoylcarnitine, 5-oxoproline, hypoxanthine, and docosahexaenoic acid) were identified as potential serum biomarkers.[355] In pancreatic cancer, a panel of 5 serum metabolites, including glutamate, choline, 1,5-anhydro-D-glucitol, betaine, and methylguanidine, was able to distinguish patients with cancer from controls.[356] Another study showed that 9 serum metabolites (histidine, proline, sphingomyelin d18:2, sphingomyelin d17:1, phosphatidylcholine, isocitrate, sphingosine-1-phosphate, pyruvate, and ceramide), combined with the carbohydrate antigen CA 19-9, were able to distinguish between pancreatic cancer and chronic pancreatitis.[357] In a study that focused on free amino acids in plasma, consistent changes were found across patients with lung, gastric, colorectal, breast, and prostate cancer compared with sex-matched and age-matched controls.[358] Interestingly, elevated plasma levels of branched-chain amino acids are an early event in pancreas cancer development (when disease is still occult) and, at the time of diagnosis, they are predictive of future tissue wasting.[210,359] In patients with osteosarcoma, serum and urinary metabolomics revealed a distinct phenotype compared with healthy patients, suggesting that downregulation of central carbon metabolism and increased glutathione and polyamine metabolism are characteristic features of osteosarcoma.[360]

Urine metabolites have also been evaluated as potential biomarkers for cancer detection. For instance, urine samples from patients with bladder cancer have a significantly lower level of citrate, 2,5-furandicarboxylic acid, ribitol, and ribonic acid compared with samples from healthy individuals.[361-363] In contrast, 2 independent studies have shown elevated taurine levels in the urine of patients with bladder cancer.[363,364] Amino acids, such as citrulline, leucine, serine, tryptophan, and tyrosine, were found to be decreased in patients with prostate cancer.[365,366] Urine metabolite analysis has also been used to detect malignancies that are not in close contact with urine. For example, patients with hepatocellular carcinoma had higher urine creatine and carnitine levels and lower citrate and glycine levels compared with a healthy cohort.[367,368] LC-MS analysis of urine samples collected from patients with lung cancer showed increased levels of amino acids tyrosine, tryptophan, and phenylalanine.[369] Modified nucleosides have also been detected in the urine of patients with a variety of cancer types.[370-372]

In addition to tissue and biofluids, exhaled breath has also been explored as a potential source of cancer biomarkers. It has been shown that volatile organic compounds in exhaled breath can be used to differentiate patients with nonsmall cell lung cancer from noncancer controls.[373-375] Sensitivity and specificity were from 72% to 90% and from 83% to 94%, respectively, which is comparable to the performance of low-dose computed tomography in lung cancer screening trials.[376]

It is important to acknowledge that a key limitation of assessing global metabolic changes in biofluids is the inability to differentiate cancer from other diseases that present with systemic metabolic alterations. Metabolomic analysis of tumor tissue is less subject to such confounding effects and, where appropriate, can be a valuable source of metabolic biomarkers that can be leveraged in early treatment phases or during disease progression when tumor tissue is available.

Discovery of Targeted Therapies That Interfere With Cancer Metabolism

A growing area of research is the use of metabolomics to uncover metabolic dependencies of cancer that can point to novel drug targets. In this section, we discuss a few examples of how metabolomics directly contributed to the discovery of new targets for precision medicine. The reader is also referred to a recent review on this topic.[377]

Although cancer drivers have been detected through genomic, transcriptomic, or proteomic approaches, understanding which cancer-associated mutations, gene expression changes, and posttranslational modifications are functionally relevant is not always straightforward. As discussed above, changes in metabolite levels reflect the activity of metabolic enzymes in cancer cells and thus could be used to help identify which alterations at the DNA, RNA, and protein levels result in functional changes in cellular activity. A classic example of this is the oncometabolite D-2-HG, which was found to be markedly elevated in cells expressing cancer-associated IDH mutations and was subsequently shown to be significantly elevated in cells, tissues, and plasma from cancers with somatic mutations in IDH.[351,378,379] These and other studies demonstrating that D-2-HG alters the activity of chromatin modifying enzymes and contributes to disease progression led to the development of drugs targeting mutant IDH, which are now in clinical trials (see Losman et al[183] and Wang et al[185]) (Table 1).

In androgen-driven prostate cancer, in which hundreds of androgen receptor-regulated genes can be differentially regulated in cancer, a combination of transcriptomics and metabolomics was used to identify calcium/calmodulin-dependent protein kinase kinase 2 as a hormone-dependent modulator of anabolic metabolism.[380] In clear cell renal cell carcinoma, multiomics data sets were compared to gain molecular insights beyond the sum of individual omics.[381] The analysis revealed crosstalk within and between phosphoproteomics, transcriptomics, and metabolomics, including known clear cell renal cell carcinoma drug targets.[381] Finally, a comprehensive analysis of expression patterns of metabolic genes across 22 diverse types of human tumors identified hundreds of tumor-specific expression changes, whereas corresponding changes in metabolite levels were used to highlight enzymes that could potentially serve as drug targets.[77]

Stable isotope tracing is another powerful tool to allow functional assessment of tumor metabolism in vivo[285] and, when combined with genomics, transcriptomics, and proteomics, can inform how altered metabolism relates to molecular drivers of cancer. Stable isotope resolved metabolomic analysis after infusion of ^{13}C-glucose into patients with lung cancer showed that tumors had increased glycolysis and Krebs cycle activity relative to noncancer tissue and also processed glycolytic metabolites differently.[333] Infusion of ^{13}C-glucose also revealed that lung cancers display metabolic heterogeneity in nutrient utilization, which could have important implications for selecting therapies that target metabolism.[83] It is noteworthy that a common theme throughout many of the studies presented here is that the best use of metabolomics is in combination with other *omics* data sets to uncover clinically relevant and actionable drug targets.

Conclusions

Although it is used less compared with the other *omics* approaches, metabolomics has the potential to significantly impact core areas of oncology, including screening, diagnosis, and therapy. However, such applications require a better understanding of how these measurements are connected to human physiology and cancer biology. In biofluids that are readily accessible clinically, most notably plasma, our understanding of which metabolites can be measured to reflect cancer status is in its very early stages. Although some inroads have been made,[210,382,383] it is still unclear to what extent a metabolite profile in plasma reveals the metabolic activity of the cancer. Additional studies conducting metabolomics experiments in fluids that harbor the cancer and connect these measurements to both metabolism and the biology of the tumor is a promising new direction. Much remains to be learned about how to interpret cancer metabolism from these measurements.

One of the challenges with metabolomics is the vast number and chemical complexity of metabolites that exist. For example, plasma metabolite composition is a manifestation of liver, muscle, and other organ-level metabolism, dietary intake, activity of the microbiome, and other factors. It is also important to recognize that metabolomics differs from other omics technology in that no one metabolomics approach can be completely comprehensive. We propose that, currently, the best use of metabolomics in research is in combination with other omics approaches and hypothesis-driven investigation to discover functionally and diagnostically relevant alterations in cancer cells. We anticipate that, as standardized protocols, affordable instruments, and user-friendly analysis platforms become more widely available, metabolomics will play an increasingly important role alongside other diagnostic and prognostic tests in the clinic and at the bedside. ■

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