Review Article

Applications of metabolomics in cancer research

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Abstract

The first discovery of metabolic changes in cancer occurred almost a century ago. While the genetic underpinnings of cancer have dominated its study since then, altered metabolism has recently been acknowledged as a key hallmark of cancer and metabolism-focused research has received renewed attention. The emerging field of metabolomics – which attempts to profile all metabolites within a cell or biological system – is now being used to analyze cancer metabolism on a system-wide scale, painting a broad picture of the altered pathways and their interactions with each other. While a large fraction of cancer metabolomics research is focused on finding diagnostic biomarkers, metabolomics is also being used to obtain more fundamental mechanistic insight into cancer and carcinogenesis. Applications of metabolomics are also emerging in areas such as tumor staging and assessment of treatment efficacy. This review summarizes contributions that metabolomics has made in cancer research and presents the current challenges and potential future directions within the field.

Keywords: Biomarkers, carcinogenesis, metabolomics

INTRODUCTION

Broadly defined, metabolism is the set of processes catalyzing the production of energy and cellular building blocks (amino acids, nucleotides, lipids, etc.) from the nutrients a cell takes up from the environment. These building blocks and the biochemical intermediates generated during their production and utilization are collectively referred to as metabolites. Metabolite levels integrate the effects of gene regulation, post-transcriptional regulation, pathway interactions, and environmental perturbations; this downstream synthesis of diverse signals ultimately makes metabolites direct molecular readouts of cell status that reflect a meaningful physiological phenotype.[1-4] Metabolomics, then, is the emerging field focused on comprehensive profiling of metabolites in a sample, whether intracellular or from circulating biofluids. The ability of metabolomics to measure high-throughput, system-wide phenotypes gives it great power in the field of oncology to further understand what is happening in cancer cells. In this review, we will focus on specific areas within cancer and carcinogenesis research on which metabolomics has been brought to bear.

COMMON METABOLIC ALTERATIONS IN CANCER

Though reprogramming of energy metabolism was only recently recognized as an emerging hallmark of cancer,[5] altered cancer metabolism was first identified almost a century ago when Warburg discovered that cancer cells primarily use anaerobic glycolysis to produce their energy instead of oxidative phosphorylation, even in the presence of oxygen – a phenomenon known as the Warburg effect or aerobic glycolysis.[6,7] Over the years, many common

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cancer mutations have been shown to support the Warburg effect.[8] AKT1 (a serine/threonine kinase), hypoxic inducing factor (HIF), and p53 (a tumor suppressor protein) together cause increased flux of glucose through glycolysis and down-regulation of flux through the tricarboxylic acid (TCA) cycle [Figure 1], thereby supporting the Warburg effect and carcinogenesis.[9-17] Loss-of-function mutations of mitochondrial enzymes succinate dehydrogenase (SDH) and fumarate hydratase (FH) also support the Warburg effect via accumulation of succinate and fumarate – metabolites that inhibit prolyl hydroxylases (PHD), a family of enzymes that tag HIF for degradation.[18-22] Thus, changes in SDH and FH support HIF accumulation, which in turn supports carcinogenesis and the Warburg effect.

Another important altered pathway in cancer metabolism is glutaminolysis, a key source of energy and anaplerotic precursors for the TCA cycle.[23] Myc, an oncogenic transcription factor, interacts with HIF to regulate several enzymes in glucose metabolism and plays an important role in glutaminolysis [Figure 1].[24,25] Besides stimulating the glutamine transporter, Myc indirectly regulates glutaminase (GLS), a mitochondrial enzyme that converts glutamine to glutamate, through transcriptional repression of the microRNAs that repress GLS.[26]

Pyruvate kinase (PK) is another common cancer signature with metabolic implications [Figure 1]. PK catalyzes phosphoenolpyruvate (PEP) conversion into pyruvate, a rate-limiting step in glycolysis. It is widely believed that during carcinogenesis, there is a change in expression of PK isoforms, from pyruvate kinase isozyme M1 towards less-active, rate-limiting pyruvate kinase isozyme M2 (PKM2),[27] potentially leading to accumulation of upstream glycolytic intermediates[8]. Additional changes in cancer metabolism prevent such accumulation (which would lead to down-regulation of glycolysis) by channeling these intermediates into branching pathways, which produces higher levels of these pathways' end products such as reduced nicotinamide adenine dinucleotide phosphate (NADPH),
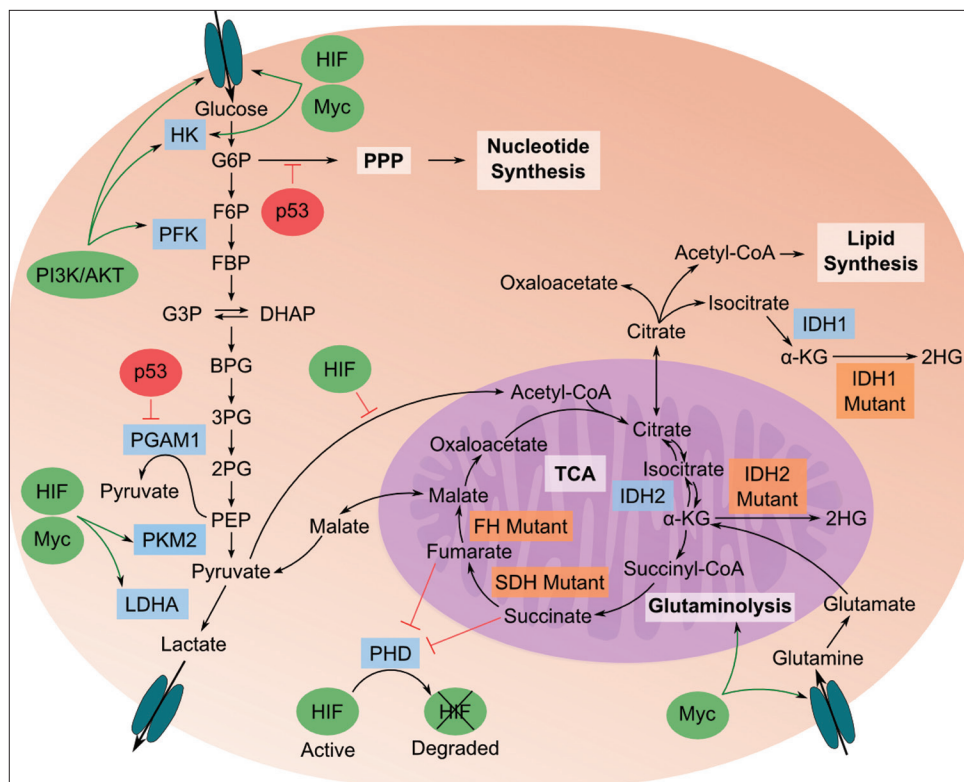

Figure 1: Illustration of important relationships between metabolome, proteome, and genome in cancerous cells. Glycolysis breaks down glucose into pyruvate, which is then fermented to lactate; pyruvate flux through the tricarboxylic acid (TCA) cycle is down-regulated in cancer cells. Pathways branching off of glycolysis, such as the pentose phosphate pathway (PPP), generate biochemical building blocks to sustain the high proliferative rate of cancer cells. Specific genetic and enzyme-level behaviors are described in the main text. Blue boxes are enzymes important in transitioning to a cancer metabolic phenotype; orange boxes are enzymes that are mutated in cancer cells. Green ovals are oncogenes that are up-regulated in cancer; red ovals are tumor suppressors that are down-regulated in cancer. Figure abbreviations: 2PG: 2-phosphoglycerate; 3PG: 3-phosphoglycerate; BPG: 1,3-bisphosphoglycerate; CoA: coenzyme A; DHAP: dihydroxyacetone phosphate; F6P: fructose-6-phosphate; FBP: fructose-1,6-bisphosphate; G3P: glyceraldehyde-3-phospate; G6P: glucose-6-phosphate; HK: hexokinase; LDHA: lactate dehydrogenase A; PFK: phosphofructokinase; PI3K: phosphatidylinositide 3-kinase

ribose 5-phosphate, and nucleic acids.[28,29]

In addition, an alternative pathway for pyruvate fermentation has recently been found that converts PEP to pyruvate through the direct phosphorylation of phosphoglycerate mutase 1 (PGAM1) without production of adenosine-5'-triphosphate (ATP) [Figure 1].[30] By decoupling energy generation from glycolysis, pyruvate production from PEP continues regardless of ATP regulation or dependence on PKM2. This continuous pyruvate production, along with glutaminolysis, accounts for the characteristically high levels of lactic acid in tumors.

METABOLOMICS AND CANCER

Analytical technology
Currently, no single analytical method can measure concentrations of all metabolites due to their significant chemical diversity. The two dominant metabolomics technologies are nuclear magnetic resonance (NMR) and mass spectrometry (MS) coupled to a separation technique. Both of these technologies and the roles they play in metabolomics are extensively detailed elsewhere,[31-33] but a brief description will be given here.

NMR
NMR provides quantitative and structural information and can measure a wide range of metabolites with little to no sample preparation. One limitation of NMR is its low sensitivity and thus higher limits of detection for metabolites. Additionally, in complex mixtures the interpretability of NMR spectra and association to specific metabolite identities can be difficult. Techniques including high-resolution NMR and high-resolution magic angle spinning NMR (HR-MAS-NMR) have been used to profile cancer metabolism in biofluids as well as tissue samples; they are particularly valuable since they do not destroy samples, allowing for parallel analysis with other techniques.[34,35] Another emerging technology, hyperpolarized NMR, has been used to characterize cancer metabolism by tracing metabolite levels *in vivo*,[36] with potential applications in clinical diagnosis or treatment of cancer.[37]

MS
MS provides semi-quantitative information with very high sensitivity, allowing the analysis of low-abundance metabolites. Many MS-based techniques require extensive sample preparation and usually can only measure specific subsets of metabolites. MS-based analyses can be broadly divided into direct injection techniques – including direct infusion MS[38] and direct analysis in real time MS (DART-MS)[39] – and separation-coupled

techniques, including gas chromatography-MS (GC-MS), liquid chromatography-MS (LC-MS) and capillary electrophoresis-MS (CE-MS). Common types of mass spectrometers include time-of-flight (TOF), quadrupole time-of-flight (QTOF), quadrupole, and orbitrap. Separation methods and MS can also be combined in series (GCxGC-MS or LC-MS/MS) to gain better separation or more structural information.

Data handling and processing
The complex raw data captured by metabolomics instruments must first be converted into human-interpretable measurements; the resulting vast datasets then require significant analysis and interpretation. Numerous data processing techniques and packages have been created for all steps of this data-processing pipeline. We refer the reader to in-depth discussions of available bioinformatics tools elsewhere.[40]

Study of carcinogenesis and cancer biology
The system-wide analyses of metabolomics allow a unique opportunity for the study of carcinogenesis and cancer biology by enabling deep investigation of targeted aspects of cancer metabolism while also allowing discovery-based analysis of metabolism writ large.

For example, nicotinamide adenine dinucleotide phosphate (NADP$^+$)-dependent isocitrate dehydrogenases 1 and 2 (IDH1 and IDH2) are commonly subject to gain-of-function point mutations in gliomas.[41] Using metabolomics, it was discovered that mutated IDH1 and IDH2 catalyze (R)-2-hydroxyglutarate (2HG), a rare metabolite, from α-ketoglutarate (α-KG) [Figure 1].[42] 2HG has been referred to as an oncometabolite because its production helps to further the cancer phenotype.[42,43] Metabolic profiling on glioma cells using LC-MS/MS and GC-MS showed that IDH1/2 mutations caused N-acetylated amino acids and TCA cycle intermediate levels to drop and biosynthetic molecules to accumulate while not affecting glycolytic intermediates.[44] The effects of IDH1/2 mutations on the metabolome were very similar to the changes caused by treating normal cells with 2HG, showing that it is the production of the oncometabolite 2HG and not the loss of IDH1/2's normal operation that causes these changes.[44]

Another (though somewhat disputed[45]) example of mechanistic insight from metabolomics is in sarcosine's putative role in prostate cancer progression. Samples from patients with benign, localized, and metastatic prostate cancer were profiled using both LC-MS and GC-MS. From this metabolic profiling, sarcosine levels were identified as increasing from benign to metastatic prostate cancer. *In vitro*, sarcosine levels were shown to directly correlate to a cell's level

of invasiveness. Further investigation showed that sarcosine is regulated by an androgen receptor and ETS gene family fusions through transcriptional control of its regulatory enzymes.[46]

A final example of metabolomics-based mechanistic insight is the recent study of extracellular metabolite profiles across the National Cancer Institute's NCI-60 cancer cell lines.[47] Glycine consumption was found to be correlated with proliferation rate in cancerous cells, but not in proliferative non-cancerous cells, suggesting cancer-specific behavior. *De novo* purine nucleotide biosynthesis was one pathway involved in the increased glycine consumption. Follow-up analysis of breast cancer gene expression data revealed that glycine mitochondrial enzyme expression correlated with cancer mortality.

Biomarkers and diagnosis

A central focus in cancer metabolomics research is biomarker discovery. Metabolites are theoretically ideal biomarkers and diagnostics because they can be easily measured from non-invasive urine or blood samples. Many groups are attempting to use metabolic profiles as biomarkers or diagnostic tools, for essentially every type of cancer, since levels of multiple metabolites can provide better classification than a single entity. For example, the diagnostic capability of a set of 113 *cis*-diol structured urinary metabolites for liver cancer resulted in a lower false-positive rate than the traditional tumor marker alpha-fetoprotein when classifying liver cancer against hepatocirrhosis and chronic hepatitis samples.[48] A representative, but necessarily incomplete, selection of applications of metabolomics for biomarker identification is discussed below, organized by cancer type.

Liver

Hepatocarcinoma, or hepatocellular cancer (HCC), has been the focus of metabolic profiling for a number of biomarker discovery and diagnostic models.[48-55] For example, serum and urine samples from HCC, benign liver tumor, and healthy patients have been analyzed using GC-MS and LC-QTOF-MS to find HCC biomarkers. Around 70 metabolites showed significant differences between cancerous samples and healthy controls.[50] Bile acids, histidine, and inosine levels had large and highly statistically significant changes between normal and HCC samples. Further analysis determined that glycochenodeoxycholic acid, glycocholic acid, taurocholic acid, and chenodeoxycholic acid differed within the HCC samples, showing correlation with liver cirrhosis and hepatitis.

Kidney

Metabolic profiling of urine samples is ideally suited to identify novel markers for early diagnosis of kidney cancer (whose incidence is currently increasing due to lack of an early diagnostic test[56-58]) due to the close interaction of urine and the kidneys.[59] Several groups have used metabolomics analysis to distinguish between cancerous and healthy urine samples, finding that acylcarnitines, quinolinate, 4-hydroxybenzoate, and gentisate were differentially accumulated.[58,60-62] Serum[38,63-65] and tissue[66,67] samples have also been used to further distinguish between kidney cancer metabolism and normal metabolism.

Breast

Beyond the aforementioned common metabolic alterations in cancer, it has been known for some time that breast cancer cells are also characterized by high levels of total choline containing compounds.[68-70] This well-known metabolic alteration has served as a gateway for significant metabolomics analysis of breast cancer.[71,72] Screening for early diagnosis has been shown to be possible using metabolomic analysis of (non-invasive) urine[73] and serum[74-76] samples, while analysis of breast tissue biopsy samples can be a useful tool as a form of secondary confirmation. For example, HR-MAS-NMR metabolomic analysis of biopsy samples has discriminated between cancerous and normal samples[77] – especially useful as it is non-destructive, allowing other analyses to be performed on the same sample.

Ovarian

A major focus in ovarian cancer metabolomics research has been in early detection, as the 5-year survival rate when caught in early stages is greater than 90%, but when diagnosed in later stages (as it is for most patients) is almost inverted.[57,78] A number of studies have attempted to use metabolomics analysis of urine or serum as an early diagnosis tool.[39,79,80] One particularly promising model used DART-MS to profile the metabolome of 44 ovarian cancer patients and 50 healthy patients through serum samples, obtaining 99% separation accuracy using a customized algorithm.[39]

Colorectal

Diagnostic biomarkers for colorectal cancer (CRC) have also been extensively explored via metabolomics.[81-86] Metabolic profiling of serum samples from cancer patients and normal controls resulted in the selection of four metabolites (2-hydroxybutyrate, aspartic acid, kynurenine, and cystamine) as the basis of an early diagnostic model. The validated model showed high sensitivity towards detecting early stage CRC.[82] Another study identified approximately 30 marker metabolites with statistically significantly different levels between normal mucosae and CRC tissue samples;

most were from pathways expected to be perturbed in cancer, such as glycolysis, lipid metabolism, and nucleotide biosynthesis.[83]

Emerging applications

Metabolomics and metastasis

Metabolomics research has shown promising results for detection of metastasis. Metabolic profiles of serum or urine samples suggest predictive capabilities for diagnosing metastases forming from gastric,[87] CRC,[88] kidney,[64,67] and breast[74,75,89] cancer. Other studies have focused on specific metastatic sites such as leptomeningeal carcinomatosis[90] and bone metastases[91], the latter of which contain higher levels of cholesterol for prostate cancer metastases when compared to other cancerous bone metastases and normal bone.

Staging of cancer

Beyond detection, metabolomics may also serve a role in distinguishing between different stages of cancer. In one study, GC-MS analysis of serum from pancreatic cancer patients was able to distinguish between Stage III, Stage IVa and Stage IVb groups.[92] Another study used GC-TOF-MS to analyze ovarian cancer samples and showed metabolic distinction of ovarian carcinomas and borderline tumors.[93] In CRC, HR-MAS-NMR profiling not only distinguished between tumor and adjacent mucosa samples, but also between the mucosa samples themselves based on the stage of their adjacent tumor.[94]

Metabolomics and treatment

An emerging field of study for metabolomics is pharmacometabolomics, the use of metabolomics to predict physiological responses for drug efficacy and/or toxicity. There are currently few pharmacometabolomics studies in oncology, but research in the area is expected to grow,[95] particularly since pharmacometabolomics is already achieving widespread attention in other fields[96-102]. In a pharmacometabolomic study of toxicity effects of capecitabine on CRC patients, lipoprotein-derived lipid levels were discovered to correlate with the intensity of toxicity, yielding predictive capabilities.[103] In another study, metabolic profiling of serum before and during chemotherapy from breast cancer patients with metastasis found that metabolite profiles from human epidermal growth factor receptor 2 (HER2)-positive patients treated with paclitaxel and lapatinib correlated with overall survival and time to progression (though the correlation did not hold across the entire population).[104]

CONCLUSION AND PERSPECTIVE

Metabolomics holds great promise for advancing the understanding, diagnosis, and treatment of cancer. The approach has already been used to uncover and verify mechanisms of carcinogenesis and proliferation, identify numerous candidate diagnostic biomarkers in biofluid and biopsy samples, and even contribute to the staging of cancers and characterization of treatment efficacy – much of this before metabolomics analysis became more widely accessible to researchers via broader establishment of metabolomics core facilities.

However, some issues must still be better addressed before metabolomics has more broad and direct clinical impact. For example, sample acquisition and preparation must be rigorously standardized in order to produce results that are reproducible enough for clinical applications, since large and fast intracellular changes in metabolite concentrations are possible during biopsies. Moreover, metabolites must be chemically identified, verified, and validated in order to see widespread adoption as clinical diagnostics (even if chemically unidentified metabolites reproducibly distinguish between experimental classes), requiring great strides in development of standard metabolite libraries or *de novo* chemical identification of unknown metabolites (both active areas of research[105-109]). Finally, more widespread clinical trials need to be conducted to provide proper validation for existing (often small sample size) biomarker research.

Future research frontiers in cancer metabolomics offer great promise, though with significant challenges. An obvious goal is to translate metabolomics measurements into deeper biological understanding of the condition, ultimately enabling better drug design and development. An increasingly popular approach to this is through the integration of multiple "omics" fields.[110,111] Integration of, for example, transcriptomic and metabolomic data has enabled deeper analysis of chemosensitive pathways[112] and breast cancer[113], and may provide further validation and understanding (and thus potential clinical applications) of discoveries.

Another prominent goal is identification of biomarkers specifically targeted toward early diagnosis. Detecting early-stage cancer, where survival rates and treatment efficacy are vastly improved, would have a transformative impact on cancer diagnosis and treatment. It remains to be seen, though, whether metabolic changes will be strong enough early indicators to be detected through non-invasive biofluid samples, or even through targeted biopsies.

Finally, the interpretation of biofluid diagnostics may prove particularly difficult. While existing analysis of blood or urine samples may find analytes that distinguish cancerous from non-cancerous samples, the metabolites detected are

often "generic" cancer-associated metabolites and may not distinguish, for example, kidney cancer from liver cancer. The commonality of dysfunctional metabolism across all types of cancer may in this case turn out to be a hindrance to interpretability and direct diagnosis; more work is needed to assess how useful such non-invasive tests could be.

Challenges notwithstanding, metabolomics is a field rife with promise to help decrease the burden and impact of all types of cancer on society.

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