New Opportunities from the Cancer Metabolome

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BACKGROUND: Metabolomics, the study of all metabolites produced in the body, which often includes flora and drug metabolites, is the omics approach that can be considered most closely related to a patient’s phenotype. Metabolomics has a great and largely untapped potential in the field of oncology, and the analysis of the cancer metabolome to identify biofluid markers and novel druggable targets can now be undertaken in many research laboratories.

CONTENT: The cancer metabolome has been used to identify and begin to evaluate potential biomarkers and therapeutic targets in a variety of malignancies, including breast, prostate, and kidney cancer. We discuss the several standard techniques for metabolite separation and identification, with their potential problems and drawbacks. Validation of biomarkers and targets may entail intensive use of labor and technology and generally requires a large number of study participants as well as laboratory validation studies. The field of pharmacometabolomics, in which specific therapies are chosen on the basis of a patient’s metabolomic profile, has shown some promise in the translation of metabolomics into the arena of personalized medicine.

SUMMARY: The relatively new approach using metabolomics has just begun to enter the mainstream of cancer diagnostics and therapeutics. As this field advances, metabolomics will take its well-deserved place next to genomics, transcriptomics, and proteomics in both clinical and basic research in oncology.

Of the omics sciences, metabolomics is one of the relative newcomers. This approach, which includes the evaluation of all of the endogenous metabolites produced by the organism as well as (on occasion) some of the metabolites of exogenous materials such as drugs, body flora, and food, is extremely powerful owing to its identification of compounds that are the true indicators of the body’s biochemistry, and by inference, the nature of life-sustaining processes. Rather than being participators in these processes, as are genes and proteins, the metabolites produced are sentinels of “what is happening” in normal physiology or pathophysiology, for example the metabolite changes that occur in response to cancer or toxicity. As such, the spectrum of altered metabolites in a disease can be considered a reflection of the abnormal processes occurring within the body, which can be useful in making inferences regarding pathology, as we discuss in this review.

In the cancer metabolome, which is defined as the entire suite of relatively low molecular weight (approximately <1500 Da) metabolites germane to cancer and includes their changes relative to a control group, metabolomic changes are a readout for normal or abnormal tumor metabolism. Because these changes can be driven by factors other than the tumor itself (e.g., drugs, meals, microflora), designing experimental and analytical models to control for these effects in the final analysis is of pivotal importance. Individual metabolite changes within the metabolome are useful for the identification of biomarkers and of novel therapeutic targets, as well as for determining which patients are more likely to respond to a particular drug (a field known as pharmacometabolomics, discussed below). Once a metabolic pathway is determined to be altered in a specific tumor or in the systemic response to it and a full in vitro and/or in vivo validation of the role of this metabolite takes place, pharmacologic inhibitors and activators of such pathways can be designed or repurposed for use as potential new chemotherapeutic agents. In this review, we discuss the field of cancer metabolomics, the techniques commonly used, and the powerful role of metabolomics in the field of cancer detection and management.

What Is Metabolomics and Why Choose this Approach?

Metabolomics can be broadly defined as the nontargeted analysis of all of the small molecule metabolites produced by the body; some but not all studies include metabolites produced by exogenous substances (e.g., from medications and gut flora) as well. These compounds are generally accepted to be ≤1500 Da and to
include peptides, oligonucleotides, sugars, nucleosides, organic acids, ketones, aldehydes, amines, amino acids, lipids, steroids, and alkaloids, and in some cases drugs or xenobiotics (1). The cancer metabolome can be considered to be those metabolites with relevance to oncologic processes and to the body’s systemic responses to the tumor, although it is somewhat unusual for reports in the literature to separate the cancer metabolome from the metabolome in general. Some investigators, especially those scientists studying the gastrointestinal tract, often include microbial products as well because these byproducts can be an indication of gastrointestinal dysfunction (2–4); other investigators include drug metabolites in the metabolome.

From the point of view of a cancer biologist studying a tissue rife with proteomic and genomic alterations, only some of which are directly contributing to the malignant phenotype, metabolomics may represent the best estimation of the pathogenesis of the disease (5, 6). Transcriptomics and genomics measure the blueprint and thus how the organism can function and proteomics measures the means by which these orders are carried out, but metabolomics measures the true real-time processes occurring within the living organism as evidenced by their ongoing metabolism (Fig. 1) (7). Whereas in other omics fields, including genomics, transcriptomics, and proteomics, tens of thousands of targets can be identified and quantified, the number of metabolites is far fewer. Indeed, the Human Metabolome Database (version 2.5) contains about 7900 metabolite entries, including both water-soluble and lipid-soluble metabolites as well as metabolites that would be regarded as either abundant (>1 μmol/L) or relatively rare (<1 nmol/L). In spite of the advances that have been made with the new technology and the release of the Human Metabolome Database (8) and other resources such as KEGG (Kyoto Encyclopedia of Genes and Genomes) (9), LipidMaps (10), and MassBank (11), the number of human metabolites under metabolomics investigation seems to be inherently low compared to the numbers involved in other omics research. Furthermore, the metabolomics approach facilitates the analysis of more than one type of sample, such as urine, blood, tissue, or cerebrospinal fluid, within the same living microorganism in a time-dependent manner. Although in theory any tissue or biofluid is amenable to metabolomics analysis, with the proper sample preparation and control patient recruitment, the most readily accessible biofluids such as urine and blood have the greatest potential for discovery of early cancer biomarkers. For these reasons, metabolomics can be considered the omics most closely associated with cancer biology, and this approach can yield abundant opportunities for applications to clinical oncology.

The Cancer Metabolome

Most of the metabolic processes in the body, such as those involving energetics and amino acid catabolism, are common to all living cells. However, it is logical that in cells that are highly proliferative or that have deregulated apoptosis, such as cancer cells, there would be some biochemical pathways that are enhanced or diminished. For example, for a cell to grow rapidly, there exists the necessity for high energy requirements as well as for the provision of membrane and other cellular building blocks. In addition, there are likely to be profound changes in metabolic pathways such as glycolysis [e.g., the Warburg effect (12)], amino acid metabolism, and fatty-acid oxidation. Although we generally think of diabetes and other endocrine disorders as metabolic diseases, these disorders largely affect one branch, or one pathway, of metabolism. However, owing to its appetite for transforming many metabolic pathways to its own advantage, which ultimately leads to its causing mortality, cancer is ideally suited for metabolomics analysis (13). Because cancer, especially in the later stages, so profoundly usurps normal metabolic processes, it can be considered the ultimate metabolic disease.

However, there are issues concerning the interpretation of changes in the cancer metabolome, largely related to the fact that cancer truly is a systemic disease. An altered cancer metabolome can be reflected in biofluids as well as in tissue, and the metabolome altera-
tions can reflect changes in the tumor itself as well as in the body’s systemic response. For example, there exist systemic responses mounted by the organism to attempt to rid itself of the disease, including the revving up of inflammation (14, 15) and the immune system (16-19). Thus, one has to be cautious when discovering and validating targets on the basis of metabolomics analysis; to attenuate a target that represents the body’s possibly successful fight against cancer would be an unfortunate development. On the other hand, cancer’s ability to mobilize and cannibalize many homeostatic systems within the body can be reflected in its metabolomic profile, further demonstrating that metabolomics is a powerful analytical tool with which biomarkers and therapeutic targets can likely be discovered.

Techniques of Metabolomics as Applied to Cancer

Techniques for metabolomics analysis must allow investigators to separate, quantify, and identify many individual metabolites present within a complicated matrix comprising a biological tissue or fluid, a daunting task given the approximately 7900 human metabolites, not including drugs, food components, and bacterial metabolites. For this reason it is clear that any one technique for measurement is unlikely to cover the entire metabolome. There exist relatively standard techniques for analysis and ultimate identification of small molecules in clinical materials. Some of these techniques, such as those based on mass spectrometry (MS), are relatively high throughput, whereas others, such as those based on NMR techniques, are relatively slow (see below). The tradeoff is that the slow techniques tend to be those that can yield more structural information. For these reasons, we have divided metabolomics identification into 2 protocols for achieving biomarker discovery, a fast and a slow “lane,” the fast lane being the ability to separate the disease state by virtue of metabolites that remain unidentified (i.e., simply by identifying significantly changed peaks or m/z values), and the slow-lane approach indicating separation of metabolites that have been chemically and structurally identified (Fig. 2) (20). Slow-lane techniques are slower because they require sometimes laborious structural and chemical identification and often require more sample quantity, but they represent the true potential of metabolomics and are required for therapeutic target identification.

The 2 general analytical approaches commonly used in modern metabolomics studies include NMR and MS (GC-MS and LC-MS) (Table 1). Each technique has its advantages and disadvantages and none is ideal for any given experiment. Frequently, a combination of different analytical technologies is used to evaluate most of the metabolites in a biological sample, and the appropriate technique has to be determined in light of the hypothesis being tested in any given study. A highly technical discussion of these methodologies is beyond the scope of this review, but has been reported elsewhere (21).

Biomarkers and Therapeutic Targets from the Cancer Metabolome

Cancer is frequently driven by a single mutation in an oncogene. It is logical to assume that such a single mutation will lead to the alteration of a single metabolic pathway. However, it is more likely that multiple pathways are affected, owing to the fact that as the cancer progresses multiple defects in biochemical pathways arise as the cancer subverts normal metabolism in an effort to survive in what can be a hostile milieu (15).

In general, metabolic requirements of cancer cells are different from those of most normal differentiated cells. To support their high rates of proliferation, cancer cells need additional nutrients and will ultimately direct those nutrients into the synthesis of new biomass, and many cancers are highly dependent on glycolysis (22). With the use of metabolomics, it is possible to exploit these various metabolic pathways by their biochemical signatures to, in theory, differentiate between cancer and noncancerous metabolic phenotypes. Given that altered metabolic pathways can actually be the driving force by which cancers may escape immune surveillance (e.g., activation of the tryptophan pathway discussed below) or gain favorable energetics (e.g., activation of the glycolysis pathway), these alterations can be tapped for biomarkers as well as to identify novel targets (19).

An illustrative example of a comprehensive metabolomics approach germane to oncology arises from recent studies on kidney cancer. Initial examination of human urine using GC-MS and LC-MS metabolomics resulted in the identification of altered concentrations of the acylcarnitines as well as metabolites that arose from tryptophan metabolism (23, 24). Using these data as a basis for further studies, we evaluated human tissue in a mouse xenograft model and were able to identify the following 3 pathways as being involved in renal cell carcinoma (RCC): fatty acid oxidation (as evidenced by altered acylcarnitines),

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3 Nonstandard abbreviations: MS, mass spectrometry; RCC, renal cell carcinoma; IDO, indoleamine-2,3-dioxygenase; tCho, total choline-containing compounds; UPLC, ultraperformance liquid chromatography.
tryptophan metabolism, and peroxisome proliferator-activated receptor-α–related pathways (19, 24).

With the use of initially nontargeted metabolomics analysis of tumor tissue, the tryptophan concentration was found to be significantly lower (0.69-fold), whereas the downstream metabolite kynurenine was significantly increased (2.78-fold) compared with controls. In light of previously described studies demonstrating that increased tryptophan metabolism is associated with both decreased proliferation of T cells and a reduced immune response mediated by the enzyme indoleamine-2,3-dioxygenase (IDO) (16, 25), these data suggest a possible mechanism for the immune escape postulated to be important in RCC patients. Validation of these data by use of inhibitors of IDO in a T-cell/RCC coculture model is currently underway. Thus, our findings that tryptophan metabolism is increased in RCC tissues and its signature is present in biofluids (Fig. 3), although consistent with what has been shown in other cancers (16, 25, 26), are notable in that derivation of these data and confirmation of the concept were all done using the technique of metabolomics.

**Early Detection and Diagnosis of Cancer**

Metabolomics has the potential to be an effective tool for early detection and diagnosis of cancer through identification of one or a group of biomarkers. In many cancer types, lipid metabolic profiles have been found to be 83% accurate when used to differentiate between cancer patients and controls by using NMR-based metabolomics of blood samples (27).

Other than kidney cancer as discussed above, it appears that the best application of metabolomics thus far in cancer diagnostics has been in breast cancer. With the use of an NMR approach, over 30 endogenous metabolites in breast tissue were identified by analyzing breast biopsy samples. Many studies of breast
cancer showed increased total choline-containing compounds (tCho), low glycerophosphocholine, and low glucose in cancer tissue compared to healthy tissue or benign tumors (28–31).

Another example of a cancer type in which metabolomics has been used is prostate cancer, which is characterized by high tCho and phosphocholine concentrations, along with an increase in the glycolytic products lactate and alanine (32, 33). Data on brain cancers also is extensive, with defined metabolomics biomarkers established from studies of brain tumor specimens exhibiting discrete ³¹H-NMR spectra (34, 35). Analysis of tCho by magnetic resonance spectroscopic imaging was able to detect breast, prostate, and brain tumors and correlated well with diagnosis via dynamic contrast-enhanced MRI (35).

Metabolomics has also been applied to ovarian cancer, for which this approach was used successfully to differentiate between healthy women and women with ovarian cancer (27). With the use of MS-based metabolic profiling of ovarian tumor tissue, 51 metabolites were found to be significantly different (P < 0.001) and could be used to discriminate between invasive ovarian carcinomas and borderline tumors (36).

Metabolomics of Radiation Therapy

Another recent example of the use of the cancer metabolome is in the field of radiation oncology. In spite of the considerable benefits obtained from the use of radiation in medicine, it is clear that there are health risks to humans exposed to radiation either accidentally or intentionally; an example of an opportunity for the cancer metabolome is to use this technique to investigate and quantify risks from radiation. Identifying noninvasive biomarkers of ionizing radiation would permit a means to discover the side effects of radiation before clinical signs appear. In addition, assessment of radiation-associated metabolite changes may facilitate the identification of novel molecular mechanisms associated with ionizing radiation as well as the DNA damage and repair response. As discussed above for kidney cancer, these findings can be useful in identifying potential targets to prevent adverse radiation events.

Biomarkers of ionizing radiation exposure have classically been those relating to DNA damage, inflammation and tissue damage, and gene expression alterations, but metabolomics has recently been used to provide a more rapid and accurate way to assess radiation effects on health. Radiation metabolomics have successfully been used to identify several biomarkers in cells (37) and in mouse urine using ultraperformance liquid chromatography (UPLC) coupled with electrospray ionization TOF-MS (38, 39), and in rat urine using GC-MS (40). The advanced analytical methods employed in these studies have provided more information on the chemical nature of biomarkers in urine. UPLC electrospray ionization TOF-MS–based metabolomics allowed effective identification of 9 urinary biomarkers of γ-radiation in rats (41). In these studies, the altered urinary biomarkers included dT, dU, and dX, N1-acetylsperrmidine, N-acetylglycosamine/galactosamine-6-sulfate, N-acetyltaurine, N-hexanoylglycine, taurine, and isethionic acid. Some of these metabolites were cross species and also were identified in the mouse (dT, dU, dX, taurine, N-hexanoylglycine) (38, 39). Although the field of radiation metabolomics has yet to be well translated to the bedside, the existing data are indeed promising and it is clear that this is an example of fertile ground for application of the cancer metabolome.

Future Potential Problems and Hurdles for Metabolomics

Although metabolomics has abundant potential for translation to the bedside, it also has limitations that may be more prominent than in other omics endeavors, so it is necessary to keep these limitations in mind.

### Table 1. Summary of the advantages and disadvantages of NMR and MS, the most commonly used methods for metabolomics analysis.

<table>
<thead>
<tr>
<th>Characterization</th>
<th>NMR</th>
<th>MS (LC-MS and GC-MS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>Large sample is required (500 μL)</td>
<td>Less sample is required (1–10 μL)</td>
</tr>
<tr>
<td>Intervention</td>
<td>Nondestructive to the sample</td>
<td>Destructive, requires derivatization (GC-MS)</td>
</tr>
<tr>
<td>Preparation</td>
<td>Simple</td>
<td>Extensive</td>
</tr>
<tr>
<td>Reproducibility</td>
<td>Very reproducible</td>
<td>Possible variation introduced by preparation</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Structural information</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Chemophysical information</td>
<td>Less information</td>
<td>More information (time separation)</td>
</tr>
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while embarking on a metabolomics study. Due to its appeal as a measure of the final processes occurring in the body, metabolomics runs the danger of being too "distal" a measure of biology. This may occur because metabolomics measures essentially the summation of many different chemical pathways and in this way may be "diluted" by confounding environmental processes, intermediate biochemical events, and exogenous compounds (e.g., bacteria, drugs). For these reasons, it is reasonable to use metabolomics as a complementary approach to other omics disciplines, so that confirmation of data from one omics by another (e.g., proteomics and metabolomics) can lead to higher confidence in the results. In one study (42), proteomics and metabolomics approaches were used to study tissue and urine, respectively, by network, pathway, and process analyses in clear cell RCC patients. Upon biomarker discovery by use of one technique (e.g., genomics, transcriptomics, and proteomics), an alternate or complementary technique was used for validation. In this manner, false discovery, which may occur with the use of only one approach, can be minimized. As in all omics endeavors, in metabolomics analysis it is essential to recruit sufficient and appropriate control patients and to have competent statistical support for both study design and data interpretation.

A special case in which metabolomics has limitations is also one of its strengths: examination of the urine metabolome. Although urinary metabolomics has resulted in promising biomarkers and targets, the fact that composition of this biofluid is complicated by diet, medications, and kidney function as well as by storage conditions (43–46) must be taken into account. These issues by no means make this biofluid unsuitable for metabolomics studies, but rather may dictate the choice of biofluid to analyze. Frequently it is impossible to obtain sufficient samples at one institution, and for this reason it may be necessary to use another institution and hence another set of collection methods. In this case, it is extremely important to be consistent in sample handling and collection to eliminate confounding variables. In general, investigators may encounter confounding analysis issues relating to consistency or disparity of collection (such as time of day and relation of sample collection to study participant’s mealtimes) and sample handling (such as collection method, time until freezing, freeze–thaw cycles) especially in metabolomics (47). Therefore, enforcement of consistent collecting protocols and storage methods is important to consider when designing a metabolomics study.

**Future Directions: Pharmacometabolomics and Personalized Medicine**

The goal of personalized medicine has been to avoid the “brute-force” approach of traditional medicine, in which drugs are given to patients on the basis of their broadly construed disease diagnosis and general manifestations, and not on their biological makeup or the molecular characteristics of the disease. Personalized therapy is likely to result in decreased frequency and intensity of adverse events because this approach directly targets the molecular defect(s). An illustrative example from general medicine, known to most healthcare practitioners, relates to the treatment of hypertension. In treatment of this disease, it is the norm to prescribe diuretics and then β-blockers, on the basis of older studies of these medications in a large population of hypertensive patients, without regard for the etiology or biology of their disease (48). Although these treatments are frequently successful, as measured by blood pressure, they are by no means tailored to the patient, and thus some patients will be overtreated,
some undertreated, and others will not respond or will have serious and perhaps unnecessary side effects. A further example, this from the clinical oncology literature, is illustrated by the use of the DNA-damaging agents. Cisplatin and doxorubicin are given to patients with a variety of cancers because it has long been assumed that damaging DNA in cancer cells will lead to regression of the tumor. Of course, this is true in many cases, but such therapeutic agents are unable to distinguish DNA of cancer cells from normal cells, which leads to serious and sometimes fatal adverse events.

Enter personalized medicine, in which the drugs to be given to patients are based on the defect that causes this disease. There is no better field for this type of practice than oncology, because most cancers are driven by one or several gene mutations, and in many cases in the modern era the process of identifying such mutations is relatively straightforward. The use of targeted therapeutics in oncology, first exemplified by the tyrosine kinase inhibitor imatinib for certain leukemias, has ushered in the era of personalized medicine. Inhibitors of other kinases, proteases, and other signaling molecules have followed, with many resulting in huge successes.

Although most of the targeted therapies are based on genetic, or in some cases proteomic, analyses of human tumors, the same strategy can be applied to metabolomics analysis (Fig. 4); in fact metabolomics can be said to more closely resemble the actual biological changes (i.e., altered metabolic pathways) that occur as a result of malignant transformation. Thus, the field of pharmacometabolomics, which not only takes into account drug effects but can also use data on drug metabolism, has enormous potential for improving personalized medicine approaches. This field has yet to burgeon, but we were able to find one example of pharmacometabolomics applications to oncology in the literature: a metabolomic profiling study of changes resulting from the exposure of MCF7 breast cancer cells to docetaxel showed varied effects at high and low doses. Although not a targeted therapy approach, it is an example of a technique that could be used to stratify patients to receive the appropriate dose of chemotherapy. In a noncancer study, a metabolomics analysis of patients taking selective serotonin reuptake inhibitors, which are used in the treatment of depression, found that the outcome of such individuals could be stratified by plasma glycine concentrations and that this metabolic event was associated with the rs10975641 single-nucleotide polymorphism in the GLDC [glycine dehydrogenase (decarboxylating)] gene. Relevant to the targets identified in kidney cancer as described above, signatures of IDO or other enzymes in the tryptophan pathway could be used to stratify patients into those with altered enzyme expression, which has obvious relevance to choice of therapy. Thus, metabolomics gives us the capability...
of distinguishing the response of an individual patient to one of these drugs on the basis of the patient’s metabolic status, so that eventually it may be possible to use a specific metabolomic profile to dictate the optimal drug for an individual cancer patient on the basis of the metabolic biology of his or her disease.

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References


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