Bioactive Food Components and Cancer-Specific Metabonomic Profiles

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Received 16 June 2010; Revised 29 September 2010; Accepted 5 October 2010

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Cancer cells possess unique metabolic signatures compared to normal cells, including shifts in aerobic glycolysis, glutaminolysis, and de novo biosynthesis of macromolecules. Targeting these changes with agents (drugs and dietary components) has been employed as strategies to reduce the complications associated with tumorigenesis. This paper highlights the ability of several food components to suppress tumor-specific metabolic pathways, including increased expression of glucose transporters, oncogenic tyrosine kinase, tumor-specific M2-type pyruvate kinase, and fatty acid synthase, and the detection of such effects using various metabonomic technologies, including liquid chromatography/mass spectrometry (LC/MS) and stable isotope-labeled MS. Stable isotope-mediated tracing technologies offer exciting opportunities for defining specific target(s) for food components. Exposures, especially during the early transition phase from normal to cancer, are critical for the translation of knowledge about food components into effective prevention strategies. Although appropriate dietary exposures needed to alter cellular metabolism remain inconsistent and/or ill-defined, validated metabonomic biomarkers for dietary components hold promise for establishing effective strategies for cancer prevention.

1. Introduction
Cancer cells exhibit unique metabolic signatures that are required for their aberrant proliferation [1]. Thus, monitoring changes in small-molecular-weight compounds (metabonomics) may represent an approach for detecting subtle shifts in tumor cell behavior [2]. Consequently, metabonomics holds promise as an effective tool for diagnosing disease progression, for identifying potential molecular targets, and for developing preventive and therapeutic agents (drugs and bioactive food components) [3, 4].

The two most effective approaches for metabonomic measures include nuclear magnetic resonance (NMR) and mass spectrometry (MS). Urine [5, 6], saliva [7], and blood plasma [8] have all been used successfully to monitor metabonomic patterns prior to and following intervention. Currently, a wealth of information about metabonomics is accumulating, thanks to highly sensitive Fourier transform ion cyclotron resonance MS (FT-ICR MS) [9, 10] and reliable candidate technologies such as stable isotope monitoring [11].

Scientists have long recognized that the discovery of novel and noninvasive biomarkers is fundamental to designing effective clinical studies, forecasting disease risk, understanding how molecular pathways are interconnected within cells and organisms, and ultimately predicting health. For example, a change in serum lactate concentration, as determined by 1H-NMR spectroscopy predicts weight gain during chemotherapy and ultimately breast cancer recurrence risk [12]. Likewise, the elevated urinary prostaglandin E(2) metabolite (11-α-hydroxy-9, 15-dioxy-2,3,4,5-tetranorpropane-1, 20-dioic acid) has been reported to predict a poor prognosis in head and neck squamous cell carcinoma patients and thus the need for more aggressive intervention approaches [13]. Unfortunately, the relationship between a given set of biomarkers and a particular pathological condition is often complicated by adjustments and adaptations, making the predictive value of such measures difficult to
establish. Metabonomic profiles of blood, urine, or tissues reflecting *in situ* status of metabolites may provide a means of identifying such biomarkers.

The annotation of metabolites identified by NMR- and MS-based experiments remains a challenge due to the diversity of study conditions. Thus, it is important to develop a standardized format that allows the scientific community to understand results from studies with widely different conditions. To facilitate this process, a working group of the Metabolomics Standards Initiative was formed in 2004 [14]. This group has established guidelines for the exchange and/or report of metabonomics data. Currently available metabonomic databases, including NIST08, PubChem, and KEGG, also help identify metabolite spectral data and structural information. An extensive review of metabonomic databases is found in a published paper [15]. Well-annotated and user-friendly databases should be able to assist in resolving unknown dietary metabolites that play a role in cancer initiation and development.

Considerable evidence points to diet as a variable that can influence cancer risk and/or tumor behavior [16]. This dietary effect may arise from its interactions with key regulatory molecules in various cancer processes, including carcinogen metabolism, hormonal balance, cell signaling, cell cycle control, apoptosis, and differentiation. The goal of this paper is to provide some insights into the metabonomic assessment of dietary effects on cancer. It is critical to evaluate whether a single or multiple dietary component(s) modulate the early transition phase from normal to cancer phenotypes, including changes in specific enzymatic activities and carbon flows.

### 2. Metabonomic Shifts Caused by Bioactive Food Components in Cancer Cells

Metabolic profiling of cancer cells represents global phenotypic changes, including those in glucose metabolism, amino acid metabolism, and fatty acid metabolism. This section discusses diet-induced alterations in basic metabolism that are associated with changes in cancer cell behavior and the monitoring of those changes using global metabonomic profiles.

#### 2.1. Glucose Metabolism

A distinct difference between normal and cancer cells lies in the way these cells utilize glucose for their survival. The energy that sustains cancer cells is preferentially derived from aerobic glycolysis, which produces significantly higher amounts of lactate from pyruvate, whereas normal cells use the aerobic tricarboxylic acid (TCA) cycle, which oxidizes pyruvate to CO₂ and water. Furthermore, proliferating cancer cells also utilize glucose for the synthesis of nucleotides through the nonoxidative pentose phosphate pathway (PPP) and *de novo* fatty acid synthesis mediated by fatty acid synthase (FAS) [1]. These differences in metabolism between normal and cancer cells are particularly interesting because they are detectable by non-invasive profiling in blood or urine and thereby can serve as clinically useful biomarkers for predicting diet-induced shifts in the metabolic switch. Potential metabonomic targets for bioactive food components are discussed in detail in the following sections and are summarized in Figure 1.

#### 2.1.1. Increased Glucose Uptake

Increased glucose uptake is recognized as a hallmark for cancer cell malignancy and serves as a basis for using ¹⁸F-deoxyglucose positron emission tomography for cancer detection. Recently, a *KRAS* mutation with aberrant cell proliferation was found to relate to the upregulation of the glucose transporter (GLUT1) gene, suggesting that genetic mutations acquired in tumor cells account for enhanced glucose uptake [17]. This change is critical for cell proliferation [18], and thus GLUT1 expression may represent a promising target for cancer prevention. A number of dietary components, including green tea polyphenolics, cinnamon polyphenol extracts, fish *n*-3 fatty acids, and vegetable flavonoids such as myricetin and quercetin, have been documented as modifiers of glucose uptake by altering GLUT1 expression [19–22]. Impressively, these effects occur in several cell lines, suggesting widespread utility of their benefits. The concentrations used, at least for some, appear physiologically relevant and therefore may occur after a simple dietary change. Although these findings provide promise for the diet-mediated prevention of cancer cell proliferation, unfortunately none of these dietary effects was examined with high-throughput metabonomic profiles. Considering the ability of metabonomics to detect changes in glucose metabolism in cancer cells [23], it is likely that these dietary effects on glucose uptake, including GLUT1 expression, can be detected using the same approach.

#### 2.1.2. Aerobic Glycolysis

The conversion of glucose to lactate in the presence of oxygen is a critical aerobic pathway that allows cancer cells to proliferate rapidly. *In vivo* expression
of the M2 isoform of the glycolytic enzyme pyruvate kinase (PK) is required for this process [24]. PK has four isozymes that are tissue specific: the L and R isoforms are expressed in liver and red blood cells, respectively, the M1 in most adult tissues and M2 in lung and embryonic cells. In normal proliferating cells, PK exists in a tetrameric form that transfers one phosphate group from phosphoenolpyruvate (PEP) to adenosyl diphosphate, yielding one molecule of adenosine triphosphate (ATP). In cancer cells, however, this enzyme is exclusively expressed as an M2 type of pyruvate kinase (PKM2), which results in a conformational change from a tetrameric to a dimeric form that possesses a low affinity for PEP and thus limits enzymatic function. This structural change was shown to be mediated through phosphotyrosine peptides or src in cancer cells [25], suggesting that oncogenic tyrosine kinases (TKs) might be promising targets for cancer prevention. Support for this conclusion comes from observations that PKM2 is released from tumors into the blood and can be monitored non-invasively. Plasma tumor PKM2 is reported to increase significantly as tumor progresses in lung cancer, qualifying it as a valuable biomarker [26]. Recently, delphinidin, an anthocyanidin that is abundant in grapes, cranberries, and pomegranates, has been reported to inhibit TK activities, including receptor TKs [27]. Additionally, isothiocyanates in broccoli have been reported to inhibit TK [28]. Overall, these findings suggest that some of the antiproliferative effects of bioactive food components may arise from their ability to convert tetrameric PKM2 to dimeric PKM2 and thereby bring about a shift in glucose flux from a nonoxidative to an oxidative pathway. Although some evidence exists that changes in glucose carbon flux from glycolysis to gluconeogenesis can be measured by the combination of LC/MS metabonomic profiling with stable isotope techniques using 13C-labeled glucose [29], these approaches have not been used for the study examining dietary effects. Thus, it is warranted to use metabonomic profiling to monitor the effects of diet or dietary components on PKM2-mediated shifts in glucose flux.

Although limited numbers of metabonomic studies have examined the cancer preventive effects of dietary components, the efficacy of the dietary supplement silybin provides some proof of principle. Silybin, extracted from a milk thistle, has been reported to significantly influence the metabolism of prostate cancer cells [30]. Quantitative high-resolution proton NMR spectroscopy was used to monitor the metabonomic profile of blood and tissue extracts in non-human animals receiving or not receiving silybin. The metabonomic profile suggested that feeding TRAMP/C57BL/6 mice with a 1% silybin-supplemented diet reduced lactate formation, increased glucose oxidation through the TCA cycle, reversed the increase in citrate use, and decreased cholesterol and phosphatidylcholine levels in prostatic tumors, which parallels earlier findings regarding the prostate cancer preventive potential of silybin in this animal model. These results suggest that non-invasive metabonomic studies can help monitor the effectiveness of cancer preventives against malignancy.

2.1.3. Increased De Novo Biosynthesis of Nucleotides via the Pentose Phosphate Pathway. Since PKM2 in cancer cells does not convert PEP to pyruvate efficiently, the upstream glucose metabolites are redirected towards a biosynthetic pathway such as PPP. Any rapidly proliferating cells, including cancer cells, utilize PPP to generate ribose-5-phosphate and the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) from glucose, which is essential for de novo DNA formation. Evidence exists that vitamin E, which is abundant in wheat germ extract and various other fat sources, inhibits nonoxidative tumor cell PPP and thereby suppresses cancer cell proliferation [31]. In leukemia patients, vitamin E was found to decrease pentose cycle substrate flow into RNA ribose, which was measured using stable isotope-labeled [1,2-13C2] glucose as the single tracer and biological MS. These changes in glucose carbon flux correlated with the cell growth-controlling and apoptosis-inducing effects of fermented wheat germ [31].

Another dietary component, genistein, which is found in soy, has also been shown to exhibit a similar effect on PPP without modulating fatty acid synthesis [32]. When MIA pancreatic cancer cells were treated with a physiological concentration of genistein (20 μM) for 3 days, the carbon flux from 13C-labeled glucose to nucleic acid ribose through the nonoxidative PPP decreased significantly [32]. These changes in PPP coincided with the cancer protective effects of either vitamin E or genistein, implying that a shift in a metabolic signature may be a useful biomarker for monitoring the efficacy of dietary components.

2.2. Amino Acid and Fatty Acid Metabolism

2.2.1. Glutaminolysis. Glucose is the major energy source in normal cells. This nutrient undergoes the metabolic switch in tumor cells from energy production to de novo biosynthesis of macromolecules, including nucleic acids. This shift increases the demand for carbon sources in cancer cells to generate ATP. It has been reported that cancer cells accumulate glutamine faster than noncancerous cells [33] and utilize it as a substrate for the TCA cycle (glutaminolysis). Ultimately, the cancer cell catalyzes glutamine to ATP and lactate. This metabolic shift is thought to be mediated by the myc oncogene [34]. It is well established that the activity of this oncogene can be modulated by various dietary components, including betaine, folic acid, genistein, and fat [35–38]. For example, supplementing the diet with betaine (1%) fed to rats developing liver tumors caused by diethylnitrosamine significantly reduced liver c-myc expression [35]. 1H-NMR spectroscopy-based metabolic profiles of plasma and urine have revealed an interaction between betaine exposure and low-density lipoprotein receptor gene expression [39]. Metabonomic profiles that explore the influence of dietary fat metabolism regulators to detect variation in c-myc oncogene expression are warranted.

2.2.2. Fatty Acid Synthase. Treatment of mouse mammary epithelial cells (HC11) in culture with 40 nM of genistein for 6 hours has been reported to significantly suppress
A variety of dietary components, including tea catechin, docosahexaenoic acid (DHA) in fish oil, β-glucan in barley and mushrooms, resveratrol in red grapes, the vegetable flavonoid luteolin, and CLA in milk, have been shown to suppress FAS expression and activity [41–46]. Treatment of the human breast cancer cell line SK Br-3, which constitutively expresses FAS, with 150 μM epigallocatechin-3-gallate (EGCG) for 24 hours resulted in a marked reduction in this enzyme activity (59% ±13%), but not its expression, compared to controls [41]. In the same study, the suppressive effect of EGCG on FAS activity was confirmed in vivo using C57BL/6J male mice. Likewise, the oral feeding of C57BL/KsJ-db/db mice with DHA (1 g DHA/100 g diet) for 4 weeks significantly reduced hepatic FAS activity accompanied by lowered levels of triglyceride [42]. Finally, feeding C57BL mice a high-fat diet supplemented with 4% barley containing a high amount of β-glucan for 12 weeks significantly reduced the hepatic FAS activity [43]. It should be possible to detect diet-induced changes in FAS with metabonomic profiling, which allows the monitoring of changes in physiologic enzyme activities using LC/MS [47].

The responsiveness of FAS to dietary components, the differential expression of this enzyme in various stages of cancer development, and its critical role in de novo lipid synthesis made it an ideal target for the dietary prevention of cancer. It has been shown that altered FAS expression brings about the signature metabolic changes in human prostate cancer. Human prostatic epithelial cells are highly unique in accumulating zinc that blocks citrate degradation. In cancer cells, however, the prostate tissue contains low levels of citrate, because it does not accumulate zinc and because most of the citrate is used for fatty acid synthesis. 1H-MRS of prostate tissues confirmed the dramatic decrease in citrate levels in prostate gland during malignancy [30]. Likewise, silibinin in milk thistle was reported to significantly decrease the expression of FAS and its transcription factor, sterol response element-binding protein 1c, as examined with 1H-MRS-based metabonomic approaches in neoplastic prostate cells [30]. The metabolic profile of prostatic tumors obtained from TRAMP mice fed a silibinin diet for 20 weeks revealed decreases in lactate and increases in citrate. Thus, a shift in the metabolic profile may help explain silibinin’s antitumorigenic effects. Fatty acid synthesis is also known to be influenced by several confounding factors, including peroxisome proliferator-activated receptors and choline availability [48]. Metabonomic profiles of tissue extracts may provide important clues about the fatty acid homeostasis that is likely to be influenced by multiple variables, including dietary habits.

### 3. Stable Isotope Technologies

The activity of bioactive food components in the body is dynamic, and thus a single point in time measurement may not lead to appropriate conclusions. Fortunately, these subtle shifts in metabolites can be traced using labels, including stable isotopes. Stable isotopes such as 13C or 15N that generate mass difference and thus can be detected by NMR or imaging technologies are widely used to trace dynamic movements, including nutrient flux, bioavailability, or kinetics in vivo [49]. Other major advantages of stable isotopes include their stability and independency, which allows long-term studies as well as the simultaneous labeling of more than one molecule on the same compound.

Although stable isotope technologies combined with NMR, especially 2D 1H total correlation spectroscopy, can be used for correlating a single isotopomer to its parental compound, the resolving power and sensitivity of this analytical technique are much less than those of MS. Stable isotope labeling, combined with various types of MS equipped with versatile ion sources such as FT-ICR, electrospray, and matrix-assisted laser desorption/ionization,
can isolate and detect the molecule of interest—even when several compounds may be crowded at one location on the profile—and thus serves as a powerful tracing technique. Representative preclinical and clinical studies using stable isotope technologies involving nutrition and cancer are provided in Table 1 [50–53]. Several excellent reviews about the merits of stable isotopes have been recently published [11, 54].

4. Metabonomics Can Detect Cancer Preventive Dietary Metabolites Generated by Microorganisms

It seems obvious that the biological functions of small-molecular-weight compounds (metabolites) are modulated by microbiota accounting for more than 100 trillion living organisms in the human gut. Gastrointestinal microbes are quick responders to dietary changes in terms of their metabolism and/or gene expression. Evidence exists that these changes may affect the utility of nutrients obtained from the diet and thereby influence human health and disease outcome [55]. The precise role of microbes in physiological metabolic pathways was investigated using a well-defined animal model transplanted with human fecal microbial communities [55]. When these animals were fed a high-fat and high-sugar diet (Harlan-Teklad TD96132), they developed increased adiposity, which can be characterized metabolically when switched to a low-fat and plant-polysaccharide-rich diet (B&K 7378000) [55]. These results suggest that microbes may either provide additional

Table 1: Preclinical and clinical nutrition studies using stable-isotope metabonomics.

| Specimen     | Analysis     | Stable isotope | Results                                                                                                                                                                                                 | Reference |
|--------------|--------------|----------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| Blood, urine | NMR, GC-MS   | $^{13}$C       | Differential glucose metabolic pathways between normal and cancer cells in lung: $^{13}$C glucose was infused to lung cancer patients and showed enhanced production of Asp and Glu via glycolysis in lung tumor tissues. | Fan et al. [50] |
| Cell extracts| GC/MS        | $^{13}$C       | The treatment of MIA pancreatic adenocarcinoma cells with 200 $\mu$M genistein for 3 days reduced the glucose (labeled with $^{[1,2-^{13}}$C] glucose) metabolism via the nonoxidative pentose pathway, which coincided with its antiproliferative effects. | Boros et al. [32] |
| Cell extracts| GC-MS        | $^{13}$C       | The altered flux in response to gluconeogenic substrates in fasting rat hepatocytes was measured with $[^{1,2-^{13}}$C]2 glucose.                                                                                  | Marin et al. [51] |
| Cell extracts| GC-MS        | $^{13}$C       | The treatment of butyrate-sensitive HT29 human colon adenocarcinoma cells with 5 mM butyrate resulted in the inhibition of glucose uptake, oxidation, and nucleic acid ribose synthesis.        | Boren et al. [52] |
| Plasma       | GC/MS        | $^{13}$C       | The hypoglycemia seen in the fasting PPARα null mouse is due to the reduced recycling of glucose carbon from lactate back to glucose.                                                                      | Xu et al. [53] |

Table 2: Metabonomic profiles of metabolites from selected bioactive food components in human blood and urine.

| Food source     | Component | Metabonomic analysis | Blood            | Urine                  | Reference                                      |
|-----------------|-----------|----------------------|------------------|------------------------|------------------------------------------------|
| Green tea       | EGCG      | HPLC-MS/MS           | EGCG             | Sulfated and glucuronidated conjugates of EGCG, hippuric acids | Del Rio et al. [58] Wang JS et al. [59].             |
| Soy             | Genistein | LC-ESI-MS/MS         | Monoglucuronides of genistein | Glucuronidated genistein | Guy et al. [60] Kano et al. [61].               |
| Cruciferous vegetables | Glucobrassicin | HPLC-MS/MS | DIM, LTr1, H1-1M | DIM | Reed et al. [62] Anderton et al. [63]. |
| Red grapes      | Piceid    | LC-MS/MS, NMR, HPLC/DAD | Sulfate and glucuronide conjugates of transresveratrol | Sulfate and glucuronide conjugates of trans-resveratrol | Burkon and Somoza [64], Boocock et al. [65]. |
| Cruciferous vegetables | Glucoraphanin | HPLC-MS, LC- (ESI)MS/MS | Sulforaphane | Mercapto-conjugates of sulforaphane (N-acetyl cysteine conjugates are major metabolites.) | Egner et al. [66], Al Janobi et al. [67]. |
| Fish, Mushroom  | Vitamin D$_2$, D$_3$ | LC-MS/MS | 25-OH vitamin D | 24,25(OH)$_2$D$_3$, 1α,24,25(OH)$_3$D$_3$ | van den Ouwehand et al. [68]. |
metabolic pathways to the host or directly modify the existing processes, resulting in altered phenotypes. Specific examples of how microbes metabolize dietary components and release cancer preventive compounds are described in detail in an earlier review [56]. With the current advances in “omics” technologies, these changes caused by human gut microbes can be directly detected by metabolomic profiles [57]. Although the application of metabolomics to microbiome projects is in its infant stage, the effects of diet on the human gut microbiome and the utilization of metabolomic analysis for its detection hold promise for future investigation.

5. Metabonomics Involving Bioactive Food Components in Clinical Studies

The current status of metabonomics that has been used for identifying and characterizing the physiological metabolites of food ingredients in humans is summarized in Table 2 [58–68]. The analysis of active food metabolites usually has been conducted with compounds known to be efficacious to human diseases. The molecules that interact with cellular components of target tissues are not food constituents but rather their metabolites that are absorbed and transported to each site of action [69–71]. For example, EGCG, which occurs in green tea, is considered to be a tumor preventive agent. Nevertheless, the prostate tissue of men who had consumed 6 cups of green tea daily for 6 weeks contained both EGCG and its methylated metabolite 4′-O-methyl EGCG. The latter has been reported to be less active than EGCG in terms of its cancer preventive action [69]. On the other hand, urolithin, a microbially generated metabolite of an active component in pomegranate juice, ellagic acid, has been found to exhibit cancer protection in various tissues, including colon, breast, and prostate [70, 71]. Thus, findings from metabonomic studies should be able to assist with clarifying how food or dietary components are metabolized in the body, possibly modulate human cell behavior, and ultimately and possibly influence human health. Currently, limited numbers of investigations exist in this area, but the science is poised to provide important new insights.

6. Future Directions

Metabonomics is the systematic study of small-molecular-weight substances that are the final products of genes and proteins. Although extensive research about genomics and proteomics is beginning to unravel the role of genes and proteins, the metabolites that dictate their characteristics for a given phenotype remain largely ill-defined. Metabolomic profiling using readily available biofluids, tissue extracts, or intact tissues may provide early clues about cellular events that influence phenotypic characteristics. Recently, there has been increased interest in cancer metabonomics and its potential for use in clinical and/or epidemiological investigations. The enriched understanding of basic mechanisms that account for shifts in the metabolome should become reality for metabonomic investigations so that effective interventions can be employed.

Although nutrition is known to modulate a number of regulatory networks involved with pathways leading to and promoting cancer, there is a dearth of studies that have examined diet as an important variable. Admittedly, there are critical issues in understanding metabolomic profiling information from nutritional studies, including the quantity and the temporal relationship of the bioactive food component consumed. Regardless, the fundamental nature of nutrition in harnessing overall cellular metabolism deserves greater attention. Controlled human intervention studies that incorporate physiologically relevant concentrations and exposures for various times are fundamental to the understanding of the utility of metabolomics as a tool for predicting phenotypic change caused by diet. Although there will be many challenges to the interpretation of these studies, their societal implications are enormous given the central role that diet has in health promotion and disease prevention, especially that related to cancer.

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