

## Expanding the Reach of Cancer Metabolomics

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### Abstract

Metabolism is again emerging as a key property that differentiates normal cells from neoplastic tissues. The coupling of this phenomenon with advanced bioanalytic methods may now open new avenues for diagnostics in cancer via discovery of chemical biomarkers. In this issue of *Cancer Prevention Research*, Montrose and colleagues apply metabolic profiling to a model of chemically induced colorectal cancer and describe the metabolomic landscape of colorectal tumors and associated biofluids in great detail. Their analysis of plasma and fecal metabolites provides inroads into the noninvasive detection of colorectal cancer using biochemical markers, as some conserved metabolic changes were altered across tumors, plasma, and feces. Meanwhile, the specific alterations identified in this study offer insights into potential metabolic drivers of colorectal cancer. For example, elevated sarcosine and 2-hydroxyglutarate were detected in these induced tumors, implicating their respective metabolic pathways and downstream interactions in colorectal cancer progression. This work highlights the potential value of cancer metabolomics for the noninvasive analysis of colorectal neoplasias while underscoring the importance of profiling diverse sample sets and metabolites in relevant cancer models to identify and validate such findings. *Cancer Prev Res*; 5(12); 1337–40. ©2012 AACR.

A complex set of integrated biochemical reactions are executed in our cells and tissues. This reaction network involves an extensive number of enzymes that interconvert diverse metabolites to carry out functions that include energy generation, biosynthesis, detoxification, protein modification, and the regulation of gene expression. As a result, our tissues harbor a complex profile of metabolites that is representative of biochemical network dynamics, and this metabolome fluctuates as a function of cellular phenotypes and tissue pathology. With the advent of new technologies that enable broad-spectrum quantitation of small molecules in cells and tissues, we are beginning to understand how changes in the metabolome correlate with specific diseases (1). In turn, this information may facilitate the discovery of potential biomarkers that can enable earlier prognoses in cancer or other diseases (2). Alternatively, these molecular signatures may provide mechanistic insights into potential therapies that target tumor metabolism (3). To accomplish these tasks, extensive analyses are required on the multitude of biologic samples available to clinicians in the hopes of identifying robust biomarkers with functional meaning.

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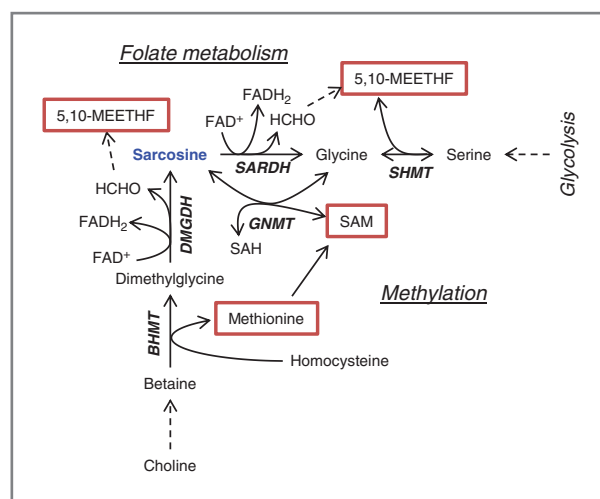
In this issue of *Cancer Prevention Research*, Montrose and colleagues profile the metabolome of azoxymethane-induced colorectal tumors and characterize parallel changes in metabolite levels within plasma and fecal samples during cancer progression (4). These analyses enabled the identification of numerous metabolites that were consistently different in plasma and feces. Several of these markers overlapped with tumor samples as well. Levels of amino acids,  $\gamma$ -glutamyl amino acids, and related compounds were significantly elevated in feces and tumors, whereas those of dipeptides were decreased. The authors also detected changes in the lipid profile of all biologic samples, although the specific lipids that varied in each sample type were generally different. The most extensive set of metabolomic differences arose when comparing normal colorectal mucosa with tumors. In addition to the above metabolites, carbohydrates (e.g., glucose, galactose, fucose) and intermediates in glucose metabolism were differentially abundant in tumors and normal tissue. Furthermore, the relative quantities of various nucleotides and associated cofactors were different in tumors.

This comprehensive analysis of metabolic changes in colorectal cancer now provides a roadmap to further elucidate the clinical potential of these signatures as potential biomarkers. Some of the changes observed in the current study were also seen in a previous comparative analysis of human colon tumors and normal mucosa (5), providing supportive evidence for this approach. The extension of metabolomics to noninvasively obtained samples is particularly exciting, as results from both fecal and plasma analyses bear similarities to those observed in tissues. The correlation of fecal heme levels with tumor

burden provides an internal means of validation, as this compound can serve as a biomarker for colorectal cancer in stool samples (6). Subsequent bioinformatics analyses may facilitate the identification of metabolomic signatures, which include combinations of metabolite changes that can be more reliably associated with tumor formation and/or outcomes (7). As the authors acknowledge, the well-controlled manner of this system afforded by consistent animal maintenance and tumor induction simplifies their analysis compared with clinical applications. However, by identifying multiplexed sets of metabolites that consistently change across tumors in studies such as this, researchers may develop more robust biomarkers for use in the clinic (8, 9). Similar approaches have been applied to correlate gene expression signatures with survival, drug responsiveness, and recurrence in colorectal and other cancers (10–12).

The relative abundances of specific metabolites in control versus tumor-bearing samples provide some insight into the molecular changes that occur during azoxymethane-induced colorectal carcinogenesis. Montrose and colleagues identified 2 interesting metabolites, sarcosine and 2-hydroxyglutarate, which were significantly elevated in colorectal tumors (4), and both compounds have recently been implicated in tumorigenesis in different cancers (13, 14). Sarcosine was first identified as a putative biomarker for metastatic prostate cancer in both urine and tumor tissue (13). Elevated levels of sarcosine correlated with prostate cancer progression to metastasis, and supplementation of this metabolite to prostate cancer cell lines induced an invasive phenotype in culture. Although the robustness of this metabolite in urine as a clinical biomarker has been challenged (15), evidence suggests that sarcosine abundance is increased in prostate tumors compared with normal tissue (16).

Sarcosine can be generated from choline-derived betaine in a reaction which forms dimethylglycine and regenerates methionine from homocysteine (17). Dimethylglycine is subsequently converted to sarcosine via dimethylglycine dehydrogenase (DMGDH), and sarcosine can ultimately be converted to glycine by sarcosine dehydrogenase (SARDH). These latter 2 reactions ultimately produce 5,10-methylenetetrahydrofolate and depend upon oxidized flavoproteins [e.g., flavin adenine dinucleotide ( $FAD^+$ )]. Notably, Montrose and colleagues observed higher levels of  $FAD^+$ , lower levels of flavin mononucleotide ( $FMN^+$ ), and expression levels of each enzyme in colorectal tumor tissue that were consistent with increased sarcosine (4). Alternatively, sarcosine can be reversibly converted to glycine and activated methionine [S-adenosyl methionine (SAM)] via glycine-N-methyltransferase (GNMT), which was also transcribed at higher levels in tumors. Virtually all metabolites along this pathway were elevated in tumor samples in their analysis (4). Therefore, it remains unclear exactly how tumors are producing this sarcosine, as numerous substrates are possible, including choline, glycine/serine and SAM, or glucose-derived serine (Fig. 1). This latter point



**Figure 1.** Sarcosine, methylation, folate, and mitochondrial metabolism. Through interconnections with cofactors and other substrates, sarcosine sits at the center of several metabolic pathways. The enzymes DMGDH and SARDH both generate 5,10-methylenetetrahydrofolate (5,10-MEETHF), which is used to generate serine from glycine via serine hydroxymethyltransferase (SHMT). DMGDH and SARDH are both mitochondrial flavoproteins, which reduce  $FAD^+$  to  $FADH_2$ . GNMT interconverts sarcosine and glycine through methylation by SAM, which generates S-adenosyl homocysteine (SAH). Methionine is regenerated through several reactions, which include betaine-homocysteine S-methyltransferase (BHMT).

highlights a mechanistic limitation of static metabolomics analyses, which often cannot decipher the directionality or rate of metabolic flux through pathways. Gene expression and metabolite abundances may or may not indicate the specific enzyme(s) or pathways responsible. Although some evidence is available relating androgen signaling to the expression of enzymes along this pathway, the functional relevance of elevated sarcosine levels remains unclear (13). The association of these metabolites with methylation and folate metabolism is clear from the pathways described earlier and provides a potential link between sarcosine and tumorigenesis (16); however, more detailed investigations are necessary to determine how this metabolite promotes cancer progression. Metabolic tracing using stable isotope-labeled compounds can better quantify fluxes and illustrate the substrates which contribute to sarcosine production (18, 19). Such analyses may provide more detailed insights into the biologic drivers of this phenotype. In the end, a combination of approaches will likely be required to fully characterize the enzymes, regulatory molecules, and substrates that contribute to increased sarcosine levels in colorectal tumors and other cancers.

In contrast to sarcosine, our understanding of 2-hydroxyglutarate function in cancer has increased greatly in the past decade. Shortly after the discovery of recurrent point mutations in  $NADP^+$ -dependent isocitrate dehydrogenases (IDH) in glioblastoma (20), several groups showed that such modifications induce neomorphic activity of IDHs in brain tumors and acute myeloid leukemia (14, 21). Rather

than interconvert isocitrate,  $\alpha$ -ketoglutarate ( $\alpha$ -KG), and  $\text{CO}_2$ , mutant IDH1 and IDH2 enzymes reductively generate (*R*)-2-hydroxyglutarate [(*R*)-2-HG] from  $\alpha$ -KG. Because of the lack of sufficient dehydrogenase capacity in human cells, tumors harboring IDH mutations can accumulate millimolar levels of (*R*)-2-HG (21). On the other hand, the (*S*)-2-HG enantiomer is generated and consumed by distinct enzymes and exhibits different biologic effects compared with (*R*)-2-HG (22). Notably, the specific enantiomer detected and mutation status of *IDH1* and *IDH2* were not delineated in this study.

Since these discoveries, several researchers have shown that (*R*)-2-HG acts as a competitive inhibitor of  $\alpha$ -KG-dependent dioxygenases in cells, and these enzymes conduct a multitude of functions in cells that include proline hydroxylation, histone demethylation, and hydroxylation of methylcytosine (leading to DNA demethylation; refs. 22–25). Elevated (*R*)-2-HG is thought to contribute to carcinogenesis by inhibiting progenitor cell differentiation through the modulation of cellular epigenetics (26). While the specific effects of 2HG in regulating colorectal cancer progression are presumably dependent on its concentration and source in tissues, endogenous or exogenous (*R*)-2-HG can induce the epithelial-to-mesenchymal transition in colorectal cancer cell lines *in vitro* (27). Although rarely occurring in colorectal cancer, the first *IDH* mutation

detected in a cancer study was identified in a colorectal tumor (28), providing evidence that (*R*)-2HG can contribute to colorectal cancer progression. While it is unclear how 2HG is generated in the azoxymethane-induced colorectal tumors investigated here, the observation that elevated 2HG is consistently upregulated during colorectal carcinogenesis is an exciting development.

Opportunities are emerging to exploit the unique metabolic phenotype of cancer for diagnostic applications and potentially clinical intervention. The potential of such an approach is captured in the experimental system described here by Montrose and colleagues, as specific changes in metabolite abundances were detected in the plasma and feces of mice bearing colorectal tumors (4). Translation to the clinic will require extensive validation. However, the ability to noninvasively obtain tissue-specific biofluids and associate chemical differences via mass spectrometry-based metabolic profiling is an important achievement that now expands our ability to characterize and observe colorectal cancer progression.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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