November 2016

HMT Newsletter

Friends and colleagues, it is time for cake!

Four years ago Human Metabolome Technologies established an independent facility in the United States geared toward bringing CE-MS metabolomics outside of the Asian/Pacific markets to world-wide distribution. Starting with one employee in 2012, HMT-America has grown to 6 employees with sales increasing every year thanks to our clients and collaborators. Soon we hope to open other offices bringing our unique metabolomics platforms to researchers across the globe.

Our recent press release on working with the NIA on Alzheimer's disease exemplifies our growing presence in the biomarker discovery and clinical research realm. Our platforms have shown remarkable translation and accuracy across multiple samples collected from distant clinical locations, a critical step towards developing strategic biomarkers for precision medicine..

We are happy to have another client testimonial this month from Dr. Debangshu Samanta (Johns Hopkins School of Medicine) writing about his systems biology approach to phenotyping cancer cells using GWAS and metabolomics. Quantifying metabolomic profiles, including isotopic labeling, is enabling scientists like Dr. Samanta to increase the understanding of cancer metabolism and to better predict drug effects against metastatic cancer.

Aside from our anniversary and recent press release, please enjoy our recent publications representing a large range of our support from cells, to isotope labeling to serum biomarker discovery. And enjoy the cake!

Sincerely,

Alexander Buko, PhD
Vice President
Human Metabolome Technologies America

Collaborator spotlight

Cancer metabolism under Hypoxia

Dr. Debangshu Samanta
Johns Hopkins School of Medicine

Ever-evolving technologies in the last 10-15 years have enabled researchers to produce an unprecedented amount of data for genome discovery - from genotyping, whole-genome sequencing and RNA. The prime challenge is to identify the key "signals" in the data that drive cancer or drug resistance for cancer patients. WGS can provide insights into known and unknown variations in approximately 95% of the individual patient's genome. Many of the GWAS studies have not found clinically useful predictors of efficacy of drugs. This is where metabolomics can provide a complimentary approach to whole genome studies in providing complete understanding of the cellular machinery.

All the small molecules (metabolites) that are present in a living system or a cell constitute the metabolome. The metabolites are result of the endogenous metabolic pathways within a cell or living organism or as result of some aberration resulting from environmental factors or some genetic aberrations or some chemical causes (drug treatment). Metabolomics technology comprehensively surveys the entire metabolome present in a single biological sample. So in a way it provides the metabolic phenotype of the cell or living organism at the basal level or due to some aberrations. As the push for better cancer therapeutics continues to rise, metabolomics will no doubt play a significant role.

Intratumoral hypoxia is the single most important feature of the microenvironment driving cancer progression. Within tumors, O_2 levels rapidly decline as distance from the nearest blood vessel increases. 52% of breast cancer tissues have a PO_2 of 2.5mmHg (0.4% O_2) or less. Median intratumoral PO_2 <10 mm Hg (1.4% O_2) is associated with decreased disease-free survival in multiple cancer types. So it is very important to get a complete metabolic phenotype of the cells under hypoxia so that the chemotherapy- radiotherapy-resistant hypoxic cells can be targeted. This also has the potential of addressing the heterogeneity that exists among cancers and to optimize/individualize metabolism based therapy.
Of course, studying metabolism under hypoxic conditions isn't without its own set of challenges, including generating sufficient cellular material for metabolomics and less protein yield, the growth inhibition of cells and developing methods of rapidly harvesting metabolites away from hypoxic conditions. As these hurdles are overcome, metabolomics performed with hypoxic cells will provide significant benefits to the cancer metabolism field. However, the complete metabolic phenotype would be obtained when the metabolomics data is combined with the stable isotope tracking methods (metabolic flux analysis). When used in concert metabolomics and metabolic flux analysis represent a powerful tool for characterizing cancer metabolism.

Glucose metabolism via the Embden-Meyerhof pathway (EMP) leads to the production of acetyl CoA, which is utilized for ATP generation through oxidative phosphorylation, and lactic acid, which is the terminal product of glycolysis. Phosphoglycerate dehydrogenase (PHGDH) diverts glucose metabolites to the serine synthesis pathway, thereby reducing production of both acetyl CoA and lactic acid. For the current project we investigated the effect of PHGDH knockdown on metabolite levels in MDA-MB-231 cells was analyzed by HMT's C-SCOPE analysis.
PHGDH knockdown was associated with increased extracellular and intracellular lactic acid levels under both non-hypoxic and hypoxic conditions. Hypoxia increased extracellular serine levels in the NTC subclone, whereas PHGDH deficiency reduced serine levels under both non-hypoxic and hypoxic conditions. Thus, serine synthesis increases in NTC cells under hypoxic conditions as a result of increased PHGDH expression. Hypoxia or PHGDH knockdown increased intracellular levels of 3-phosphoglyceric acid, 2-phosphoglyceric acid, phosphoenolpyruvic acid, and pyruvic acid. Hypoxia decreased intracellular levels of 6-phosphogluconic acid, the G6PD reaction product, which is consistent with decreased G6PD expression under hypoxia. Taken together, these data suggest that under hypoxic conditions in MDA-MB-231 cells, glucose is shunted to the serine synthesis pathway and diverted away from the pentose phosphate pathway.

Reference:
"PHGDH Expression is Required for Mitochondrial Redox Homeostasis, Breast Cancer Stem Cell Maintenance and Lung Metastasis"
Samanta D. *et al.*, *Cancer Research*, **76**, *pp*. 4430-4442, 2016.

Biography:

Dr. Samanta earned his PhD in Cancer Biology from Vanderbilt University, where his thesis was focused on how smoking induces tumorigenicity and therapeutic resistance in lung cancer. For his postdoctoral work, he has studied regulatory mechanisms of cancer stem cell population in Dr. Gregg L. Semenza's lab at Johns Hopkins School of Medicine, focusing on the contribution of metabolism to cancer stem cell phenotype.

HMT Updates

News release

HMT begins new contract with NIA/NIH to study brain metabolomic profiles in Alzheimer's disease.

Human Metabolome Technologies (HMT) has been awarded a contract to work with the National Institute on Aging (NIA) on a study looking at the mechanisms associated with Alzheimer's disease (AD). It is hoped that prevention and treatment of AD can be accelerated by combining clinical, genetic, epidemiologic, and imaging research with comprehensive metabolic phenotyping of human brain tissue.

Featured articles

Hyaluronan production regulates metabolic and cancer stem-like properties of breast cancer cells via hexosamine biosynthetic pathway-coupled HIF-1 signaling.

Chanmee T. *et al., J. Biol. Chem., in press.*

Cancer stem cells (CSCs) represent a small subpopulation of self-renewing oncogenic cells. Like many other stem cells, metabolic reprogramming has been implicated to be a key characteristic of CSCs. However, little is known on how the metabolic features of cancer cells are controlled to orchestrate their CSC-like properties. We recently demonstrated that hyaluronan (HA) overproduction allowed plastic cancer cells to revert to stem-cell states. Here, we adopted stable isotope-assisted tracing and mass spectrometry profiling to elucidate the metabolic features of HA-overproducing breast cancer cells. These integrated approaches disclosed an acceleration of metabolic flux in the hexosamine biosynthetic pathway (HBP).

Changes in energy metabolism due to acute rotenone-induced mitochondrial complex I dysfunction - An in vivo large animal model.

Karlsson M. *et al., Mitochondrion, in press*

Metabolic crisis is a clinical condition primarily affecting patients with inherent mitochondrial dysfunction in situations of augmented demand. To model this, ten pigs received an infusion of rotenone, a mitochondrial complex I inhibitor, or vehicle. Clinical parameters, blood gases, continuous indirect calorimetry, *in vivo* muscle oxygen tension, *ex vivo* mitochondrial respiration and metabolomics were assessed.

Exposure of C57BL/6J mice to long photoperiod during early life stages increases body weight and alters plasma metabolomic profiles in adulthood.

Uchiwa T. *et al., Physiol. Rep.*, **4**, e12974.

Perinatal photoperiod is an important regulator of physiological phenotype in adulthood. In this study, we demonstrated that postnatal (0-4 weeks old) exposure of C57BL/6J mice to long photoperiod induced persistent increase in body weight until adulthood, compared with the mice maintained under short photoperiod.

Serum metabolomics analysis for early detection of colorectal cancer.

Uchiyama K. *et al., J. Gastroenterol., in press*

Although colorectal cancer (CRC) is one of the most common causes of cancer mortality, early-stage detection improves survival rates dramatically. Because cancer impacts important metabolic pathways, the alteration of metabolite levels as a potential biomarker of early-stage cancer has been the focus of many studies. Here, we used CE-TOFMS, a novel and promising method with small injection volume and high resolution, to separate and detect ionic compounds based on the different migration rates of charged metabolites in order to detect metabolic biomarkers in patients with CRC.

HMT is a leading company providing metabolomic profiling based on unique and high performance CE-MS technology. We complete over 400 projects a year and our technology has contributed to the advancement of research in a variety of scientific areas.

Edited by Takushi Oga, PhD

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