HMT Newsletter

Friends and Colleagues,

August can be a slow month for research with academic and summer vacations, however, no rest for HMT. This month's release of an **enzymatic kit for PEA** for **major depressive disorder (MDD)** marks the start of what we hope is a long list of commercial biomarkers stemming from our original research. This kit will be used for large scale validation of PEA for MDD. Since PEA was first observed using our CE-MS platform as an unknown metabolite using our **Advanced Scan**, I felt it appropriate to talk this month about the strength of our Advanced Scan platform. With our high resolution and accuracy mass measurements, new discoveries continue to be made using this capability across different areas of metabolomic research.

Our key publications this month reflect our focus on central energy metabolism, measured by another popular platform, **C-SCOPE**. With C-SCOPE, we quantify 116 metabolites in energy metabolism pathways perfect for **mitochondrial dysfunction** and biochemistry research.
For those of you who joined us for Laura's **webinar** on **F-SCOPE**, we thank you for your many questions and hope you have a clearer understanding of our ability to measure isotopic distributions for metabolite flow. If you missed her webinar, we plan to have it available on our website and on YouTube shortly.

Sincerely,

Alexander Buko, PhD
Vice President
Human Metabolome Technologies America

Application note

HMT Advanced Scan: The Iceberg Effect

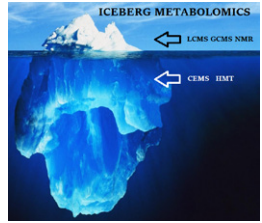
Dr. Alexander Buko
Vice President
Human Metabolome Technologies America

Advanced Scan, a global screening of metabolites including unknown compounds, allows us to work with our clients to create novel discovery and capture a larger chemical space or metabolomic window for biomarker discovery.

The Iceberg Effect

What if you could see something others could not see? What if you had the technology to discover novel molecules that were biomarkers of disease or bacterial infection? What if you could uncover new pathways and create new ideas from making observations yet unseen? Look beneath the usual suspects? This is called the Iceberg Effect.

At HMT we are using CE-MS to open up a new window to the metabolomics world by using a separation that is so unique and so very different than what has been the normal for metabolomic profiling. For years the majority of metabolomics has been accomplished using HPLC-MS, GC-MS and NMR to monitor and discover a changing metabolome for many applications - industrial, academic, and biomedical.
A few thousand metabolites, many of them lipids or hydrophobic metabolites, have been identified and many of those quantitated in various biofluids or tissues.



However, we are at a crossroads when it comes to new discovery. After many years of measuring metabolomics (and other omics) much science has been accomplished, but still many unanswered questions exist. Where are the biomarkers, indicators of disease progression, prognostic markers of pending and chronic diseases? How can we best use the bounty of information we all have been accumulating for the past 10 to 15 years?
Part of the answer is the systems biology of using a combination of metabolomic profiling with other measurements and other analytes (DNA, RNA, Protein), clinical phenotypes, clinical measurements (BMI, Cholesterol, Uric acid...). The other part of the answer is to look for metabolites yet undiscovered. This is where Advanced Scan from HMT creates new discoveries.

Advanced Scan builds on Basic Scan
"Advanced Scan" is HMT's untargeted analysis measuring all metabolites regardless of identity, and has broader target space compared with "Basic Scan", screening of identified and tentatively identified metabolites.
At first you might consider Advanced Scan as just a listing of unknown metabolites, those not in our annotation library. However, after collecting data and analyzing it, you will find there is much more here than just unidentified peaks. We can postulate or identify a molecular formula using exact mass and these peaks often represent novel discovery, metabolites yet undiscovered.

The Power of CE-MS allows for new discoveries
Due to the specificity of CE to observe and measure polar, charged or ionic small molecular weight metabolites, CE is capable of discovering many new metabolomic signatures. While LC-MS methods will have many unidentified metabolite peaks as well, many of these are lipophilic, larger metabolites that most likely are modified related lipids. The majority of unidentified peaks in our advanced scan are under a molecular weight of 300, too small for typical LC-MS untargeted discovery methods, hence many of these unknown metabolites are unseen or poorly seen by LC-MS, GC-MS and NMR methods, and therefore unique to CE-MS.
In general, the majority of identified metabolites found in the literature and common public databases like MetLin and HMDB were discovered or quantitated by HPLC and/or LC-MS hence those metabolites more likely to be observed by capillary electrophoresis or non HPLC methods are largely missing from these libraries.
Our mass accuracy of 0.001 amu allows us, in many cases, to propose unique molecular formulas. Although we have an extensive metabolite library, many unidentified peaks in our Advanced Scan do not match the molecular masses or formula for metabolites listed in our or public databases (pubmed, HMDB, MetLin, Bigg, Metscyc, ECMDB etc.) suggesting these are uniquely identified by CE-MS.
Many of these novel metabolites could be from the microbiome while others may be new endogenous metabolites indicative of homeostasis, toxicity, aging or chronic disease. Molecular formulas and MS fragmentation data tell us something about the origins of these new metabolites.

Confirming identify of unknowns
Our biomarker pipeline contains lead metabolites that were first discovered as unknowns. The process starts with our ability to measure metabolite peaks with 0.001 amu accuracy. This allows us in many cases to postulate a molecular formula. Any fragment ions and the migration time may also provide clues to identity. Once identity is postulated, standard compounds are obtained to match to the unknown. This is a process that is individualized each time depending upon the concentration and uniqueness of the unknown.

With HMT, we have designed our platforms for success and provide many options to create the right discovery tools for your research goals. I continue to look forward to applying our CE-MS based methods, including Advanced Scan, to new pre-clinical and clinical projects, building up our annotation library and delivering new possibilities to the research world in industry and academia.

Dr. Alexander M. Buko

VP, Human Metabolome Technologies America, Inc.

Dr. Alexander M. Buko is completing his 3rd year with Human Metabolome Technologies, following a 30 year career in pharmaceutical and biotechnology, leading groups in translational medicine.
While at Abbott and Biogen, Alex's groups used CE-MS, LC-MS, GC-MS and NMR to discover and verify biomarkers and new drug targets.

HMT Updates

News release

HMT release MDD diagnostic assay kit in the collaboration with G-TAC

On August 10, Human Metabolome Technologies, Inc. (HMT) announced the release of a clinical enzymatic assay kit for PEA (Phosphoethanolamine), as a diagnostic marker for major depressive disorder (MDD), in the collaboration with G-TAC Co., Ltd. (G-TAC).
G-TAC was founded by M3 Inc. providing clinical information for precision medicine to medical providers and patients. In this collaboration, HMT will provide the PEA enzymatic assay kit to hospital and laboratory clinics for research purposes towards validation of PEA as a biomarker for MDD with G-TAC providing information and marketing. The release of the kit will start in Japan September this year.

Featured articles

Cell-permeable succinate prodrugs bypass mitochondrial complex I deficiency.

Ehinger JK., *et al., Nat Commun*., **7**:12317.

Mitochondrial complex I (CI) deficiency is the most prevalent defect in the respiratory chain in paediatric mitochondrial disease. This heterogeneous group of diseases includes serious or fatal neurological presentations such as Leigh syndrome and there are very limited evidence-based treatment options available. Here we describe that cell membrane-permeable prodrugs of the complex II substrate succinate increase ATP-linked mitochondrial respiration in CI-deficient human blood cells, fibroblasts and heart fibres.

The ERK signaling target RNF126 regulates anoikis resistance in cancer cells by changing the mitochondrial metabolic flux.

Yoshino S., *et al., Cell Discovery*. **2**: 16019.

Loss of anchorage to the extracellular matrix leads to apoptosis (anoikis) in normal cells, but cancerous cells are usually resistant to such stress. Here we report the pivotal role of an E3 ubiquitin ligase, ring-finger protein 126 (RNF126), in the resistance of cancer cells to the stress associated with non-adherent conditions. Non-adherent cancer cells exhibited increased flux through the tricarboxylic acid cycle via increased conversion of pyruvate to acetyl-CoA.

Mitochondrial Mg homeostasis decides cellular energy metabolism and vulnerability to stress.

YamanakaR., *et al., Sci. Rep.*, **6**: 30027.

Cellular energy production processes are composed of many Mg dependent enzymatic reactions. In fact, dysregulation of Mg homeostasis is involved in various cellular malfunctions and diseases. Recently, mitochondria, energy-producing organelles, have been known as major intracellular Mg stores. Several biological stimuli alter mitochondrial Mg concentration by intracellular redistribution. However, in living cells, whether mitochondrial Mg alteration affect cellular energy metabolism remains unclear. Mgtransporter of mitochondrial inner membrane MRS2 is an essential component of mitochondrial Mg uptake system. Here, we comprehensively analyzed intracellular Mglevels and energy metabolism in Mrs2 knockdown (KD) cells using fluorescence imaging and metabolome analysis.

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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