HMT Newsletter

Dear Researchers,

We at Human Metabolome Technologies continue to improve our technology to meet the expectations of those researchers who are leading metabolic studies. While steady state metabolomics provides insightful snapshots of cellular metabolic pools where comparisons among samples can often reveal differences in glycolytic output or proliferation rates, however, due to the fluidity of metabolic processes, often subtle changes in preferred metabolic pathways can be missed. In contrast to steady profiling, stable isotope flux analysis provides insight into the direction and flow of those isotopically labeled atoms, typically carbon or nitrogen. Due to HMT's high mass resolution technology, we can accurately detect and quantify isotopomer distributions using our F-SCOPE platform. In this newsletter, we are pleased to introduce some of our recently published research articles.

Sincerely,

Tsutomu Hoshiba
CEO
Human Metabolome Technologies America

HMT Updates

Conference and Exhibition Information

FDA Center for Biologics Evaluation & Research (CBER) vendor day
July 10, 2015, Silver Spring, Maryland

HMT will present our services and recent applications at exhibit space. Please share your research goals with us if you attends the event.

Our Recent Applications

Heavy isotope flux analysis, which use substrates including 13C or 15N, provides insight into the direction and flow of those labeled markers, typically glucose or glutamine.

For glycolysis, lactate, the final product, is considered as an indicator of glycolytic output, but information from steady state metabolic levels is limited because it reveals only the balance between production and consumption. Labeling analysis using 13C glucose can quantify the production derived from glucose which is a direct indicator of pathway activity.

Heavy isotope analyses can also often be used to distinguish between multiple enzymatic reactions that result in the same product. For example, citrate can be formed from the traditional oxidative reactions of the TCA cycle, however, it can also be formed from a reversal of the cycle via reductive carboxylation. Unlabeled steady state measurement cannot definitively determine the directionality of the cycle. However, by using uniformly 13C labeled glutamine, a clearer picture can emerge as one carbon will be lost as C02 via the oxidative pathway, while all 5 carbons will be retained via the reductive pathway. Therefore, analyzing the change in percentage of 4 or 5 labeled carbons in citrate can give clues to changes in the directionality of the TCA cycle.

These approaches can identify specific sensitive reactions for potential therapeutic intervention and mechanism of action studies.

Signaling through the Phosphatidylinositol 3-Kinase (PI3K) / Mammalian Target of Rapamycin (mTOR) Axis is Responsible for Aerobic Glycolysis mediated by Glucose Transporter in Epidermal Growth Factor Receptor (EGFR) -mutated Lung Adenocarcinoma.

Hideki Makinoshima *et al., The Journal of Biological Chemistry, in press*, 2015

Oncogenic epidermal growth factor receptor (EGFR) signaling plays an important role in regulating global metabolic pathways including aerobic glycolysis, the pentose phosphate pathway (PPP) and pyrimidine biosynthesis. However, the molecular mechanism by which EGFR signaling regulates cancer cell metabolism is still unclear. To elucidate how EGFR signaling is linked to metabolic activity, we investigated the involvement of the RAS/MEK/ERK and PI3K/AKT/mTOR pathways on metabolic alteration in lung adenocarcinoma (LAD) cell lines with activating EGFR mutations.

Quantification of folate metabolism using transient metabolic flux analysis

Philip M Tedeschi *et al., Cancer & Metabolism*, **3**; 6, 2015

Systematic quantitative methodologies are needed to understand the heterogeneity of cell metabolism across cell types in normal physiology, disease, and treatment. Metabolic flux analysis (MFA) can be used to infer steady state fluxes, but it does not apply for transient dynamics. Kinetic flux profiling (KFP) can be used in the context of transient dynamics, and it is the current gold standard. However, KFP requires measurements at several time points, limiting its use in high-throughput applications. Here we propose transient MFA (tMFA) as a cost-effective methodology to quantify metabolic fluxes using metabolomics and isotope tracing.

Other Publications

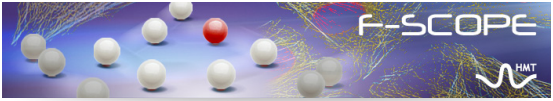
SHMT2 drives glioma cell survival in ischaemia but imposes a dependence on glycine clearance.
Dohoon Kim *et al., Nature*, **520**, *pp*. 363-367, 2015

Suppression of Microbial Metabolic Pathways Inhibits the Generation of the Human Body Odor Component Diacetyl by Staphylococcus spp.
Takeshi Hara *et al., PLoS One*, **9**, e111833, 2014

Metabolomic profiling rationalized pyruvate efficacy in cybrid cells harboring MELAS mitochondrial DNA mutations.
Kenjiro Kami *et al., Mitochondrion*, **12**, *pp*. 644-53, 2012

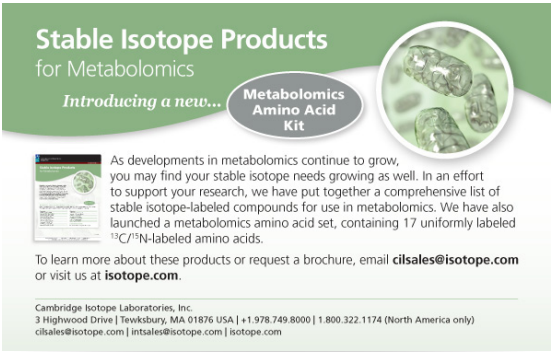
Nrf2 redirects glucose and glutamine into anabolic pathways in metabolic reprogramming.
Yoichiro Mitsuishi *et al., Cancer Cell*, **22**, *pp*. 66-79, 2012

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.



Recommended Isotope Substrate Supplier

Cambridge Isotope Laboratories, Inc. (CIL) is the world's leading producer of stable isotopes and stable isotope-labeled compounds. With over 400 employees and laboratories in four countries, CIL specializes in the process of labeling biochemical compounds with highly enriched, stable isotopes of Sugar, Organic acid, Amino Acid, etc.



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