HMT Newsletter

Friends and Colleagues,

It is now June. Spring time. A time of change in the weather, school graduations and summer vacations. Change is also the keyword for this month's newsletter.

Our introductory message is from our own Dr. Laura Shelton, who will discuss in vivo metabolism. Laura discusses our initial work measuring the course of C13-labeled glucose administered to a mouse xenograft model using HMT's F-Scope platform.
In our publication area we have three articles this month reflecting change. The transformation of glutamate in proliferating and quiescent oncogenic cells (Coloff), the metabolic reprogramming in cancer stem cells (Sato) and how differentiating factor -1 (DIF-1) lowers glucose in a diabetic model (Kawaharada). Our metabolomic profiling platforms measure change. Quantitation, sensitivity, accuracy and precision are the hallmarks of our CE-MS based platforms.
As does metabolism change, so does our company. Earlier this year I mentioned how we opened our new biomarker division, focused on the development of clinical biomarkers. This month, we build on that with the announcement of our new collaboration with M3 Inc., a global informatics institution driving new technologies, physician support and clinical biomarker development.

Sincerely,

Alexander Buko, PhD
Vice President
Human Metabolome Technologies America

Application note

HMT F-SCOPE: 13C labeling analysis solution - Progress to understanding in vivo metabolomics -

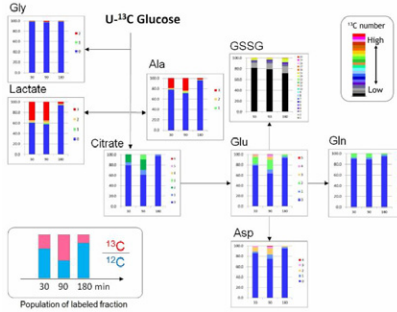
Dr. Laura Shelton
Scientific Project Coordinator
Human Metabolome Technologies America

One key to understanding cancer metabolomics lies in the ability to accurately replicate the natural environment of the tumor in the lab.
In vitro culturing of cancer cell lines is an easy way to initially understand the metabolic needs of specific cell lines and gene mutations. However, too often, drug targets identified *in vitro*, do not translate *in vivo* or in the clinic. The cellular and genetic heterogeneity and feedback mechanisms of the natural environment *in vivo* is missing in the *in vitro* setting. Therefore, it is important to also be able to accurately study real time metabolomics in the natural environment of the tumor.

C13 isotope analyses are a very useful tool for following the fates of nutrients inside the cell. *In vitro* experiments are easy to perform and can yield valuable insights. The push towards *in vivo* C13 labeling will allow researchers to confirm the cells preferred metabolic pathways and better understand druggable targets. The *in vitro* environment is lacking in the intricacies of a tumor environment. As shown in "*Metabolic Heterogeneity in Human Lung Tumors*" by Hesley et. al., *in vivo* analyses often can reveal a switch in metabolic phenotypes due to limited availability of glucose or oxygen, things that are hard to accurately replicate in the *in vitro* environment.

HMT is committed to providing our unique C13 analyses for cultured cells, as well as, human tumors *in vivo*. As a proof of concept (POC) experiment, we performed a time course analysis injecting a single dose of C13 glucose intraperitoneally into a mouse and analyzed subcutaneous tumors at 30, 90, and 180 minutes post injection for C13 incorporation into targeted metabolites.

In the figure below, we observed rapid labeling of lactate that dropped off by 180 minutes. The number of labeled carbons is marked by the legend, illustrating in red, that all 3 lactate carbons were isotopically labeled with C13. Glycine is only labeled in a small portion (~2%), though it is well above the natural abundance. Glycine synthesis may be too slow to acquire a significant portion of the C13 glucose when it is injected as a single dose or these cells may not actively synthesize glycine, while both alanine and aspartate contained a significant level of label. We also see that the labeled carbons are entering the TCA as 2 carbon labeled acetyl CoA, thus resulting in 2 carbon labeled citrate (dark green). We also notice that the cells are actively synthesizing both glutamate, presumably for glutathione synthesis, as well as glutamine, again illustrated by the significant portion of 2 carbon label in these metabolites as they will be produced from α-ketoglutarate from the TCA. Overall, we saw C13 incorporation into selected glycolytic intermediates, TCA cycle, amino acids, glutathione, and ATP. Future studies will focus on increasing the level of detection of incorporated C13, adding additional metabolites, and investigating the metabolic consequences of various anti-cancer agents. HMT also understands that every experiment is different, and we are working on developing additional labeling protocols based on the goals of the experiment.



As the push for better cancer therapeutics continues to rise, metabolomics will no doubt play a significant role. Metabolic therapies themselves are subject to varying responses, sometimes within the same cell line based only on whether or not the cells are tested *in vitro* or *in vivo*. It is imperative that we assess our systems both *in vitro* and *in vivo* for a better understanding of how these cells thrive, progress, and respond to cancer therapies.

Reference

"Metabolic Heterogeneity in Human Lung Tumors." Hensley C. T., et. al., *Cell*. **164**, *pp*. 681-694, 2016.

HMT Updates

News release

HMT announced capital alliance with M3 Inc.

On May 24th, HMT has announced the capital alliance with M3 Inc. (Headquarters: Tokyo, Japan; CEO: Itaru Tanimura; "M3" below) to advance personalized medicine through the development of innovative biomarkers. M3 provides various services for the 250,000+ physician members via the "m3.com" website designed for medical professionals in Japan. Furthermore, consulting services in research and development of new drugs and medical devices are provided via subsidiaries Integrated Development Associates Co., Ltd. and POC Clinical Research Inc. HMT and M3 have entered this capital and business alliance in conclusion of the joint objective to advance personalized medicine through the excavation of innovative biomarkers, by utilizing M3's comprehensive resources to maximize the value of HMT's biomarker development platform.

Featured articles

Differential Glutamate Metabolism in Proliferating and Quiescent Mammary Epithelial Cells.

Coloff JL., *et al.*, *Cell Metab.* **23**, *pp.*867-880.

Mammary epithelial cells transition between periods of proliferation and quiescence during development, menstrual cycles, and pregnancy, and as a result of oncogenic transformation. Utilizing an organotypic 3D tissue culture model coupled with quantitative metabolomics and proteomics, we identified significant differences in glutamate utilization between proliferating and quiescent cells.

Spheroid cancer stem cells display reprogrammed metabolism and obtain energy by actively running the tricarboxylic acid (TCA) cycle.

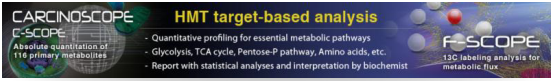
Sato M., *et al.*, *Oncotarget, in press.*

The Warburg effect is a metabolic hallmark of cancer cells; cancer cells, unlike normal cells, exclusively activate glycolysis, even in the presence of enough oxygen. On the other hand, intratumoral heterogeneity is currently of interest in cancer research, including that involving cancer stem cells (CSCs). In the present study, we attempted to gain an understanding of metabolism in CSCs that is distinct from that in non-CSCs.

Oral administration of Dictyostelium differentiation-inducing factor 1 lowers blood glucose levels in streptozotocin-induced diabetic rats.

Kawaharada R., *et al.*, *Life Sci., in press.*

Differentiation-inducing factor 1 (DIF-1), originally discovered in the cellular slime mold Dictyostelium discoideum, and its derivatives possess pharmacological activities, such as the promotion of glucose uptake in non-transformed mammalian cells
in vitro. Accordingly, DIFs are considered promising lead candidates for novel anti-diabetic drugs. The aim of this study was to assess the anti-diabetic and toxic effects of DIF-1 in mouse 3T3-L1 fibroblast cells *in vitro* and in diabetic rats *in vivo*.



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

Human Metabolome Technologies America
24 Denby Road, Suite 217, Boston, MA 02134, USA | p. 617-987-0554 | f. 617-902-2434
hmtamerica@humanmetabolome.com | humanmetabolome.com/en