

HMT Newsletter

Friends and Colleagues,

Altered metabolism is now a "hallmark" of cancer. Cancer metabolism refers to the alterations in cellular metabolism pathways that are evident in cancer cells compared with most normal tissue cells. This month's newsletter starts with Jeff Kremer's work in Brian Van Tine's lab at Washington University, St. Louis, studying ASS1 deficient sarcoma cells. Cancer metabolic profiling continues to be one of our focus areas. We will also be presenting a booth exhibit at AACR in April at the Washington Convention Center. Later in the month we will also present at the Japanese Medical Society of America (JMSA) in New York City.

Lastly, our featured articles this month expand beyond cancer metabolism to heart disease and parasite infection. Please enjoy this month's topics and, as always, share with friends and colleagues.

Sincerely,

Alexander Buko, PhD
Vice President
Human Metabolome Technologies America

Special Report

Metabolomics identifies multiple synthetic lethal therapies for ASS1 deficient cancers

Jeff Kremer
Washington University in St. Louis

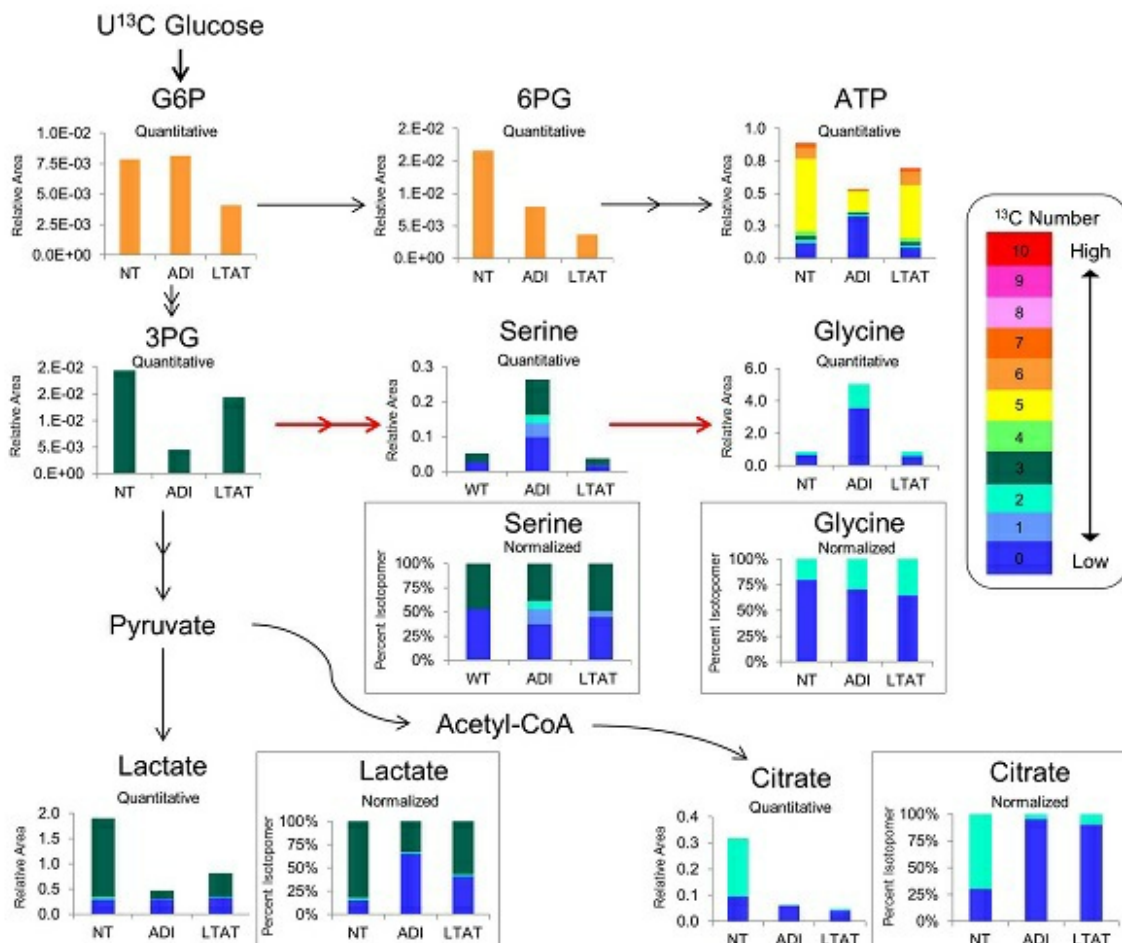
Identifying and understanding cancer metabolism is a rapidly evolving opportunity for cancer treatment. Tumorigenesis is associated with unexpected alterations in cellular metabolism that arise from the need for cancer cells to generate the energy and biomass that is necessary to maintain cell growth and proliferation. As a result, cancer metabolism represents the greatest opportunity for the next generation of cancer therapeutics after the immunotherapy era.

As our understanding evolves, even the most basic principles of cancer metabolism will be further defined. For example, recent papers have demonstrated that as cancer develops it makes multiple alterations in amino acid metabolism including the upregulation of glutamine

metabolism, increased rates of serine biosynthesis, and loss of key enzymes involved in the biosynthesis of asparagine as well as arginine. Many of these alterations have shown promise for the development of metabolic based therapeutics.

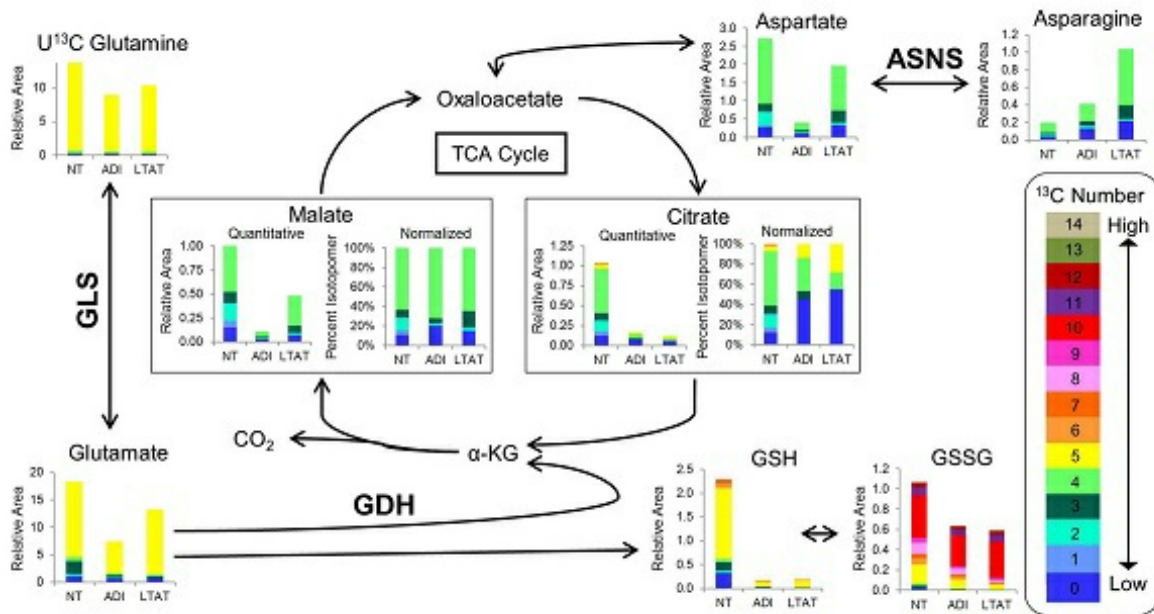
Previous research in the Van Tine laboratory has demonstrated that approximately 90% of sarcomas, a group of approximately 100 rare tumors of mesenchymal origin, lack expression of argininosuccinate synthetase 1 (ASS1), the enzyme responsible for catalyzing the penultimate step of arginine biosynthesis, loss of ASS1 forces cells to rely on extracellular arginine for continued cell growth and proliferation and sensitizes cells to therapeutics that degrade extracellular arginine. PEGylated arginine deiminase (ADI-PEG20) degrades arginine into citrulline and ammonia and has been shown to induce autophagy in sarcoma cells, but not cell death. ASS1 reexpression occurs upon prolonged ADI-PEG20 treatment, driving the need for the combination of arginine deprivation with therapeutics targeting the response pathways to induce cytotoxicity before metabolic rewiring leads to acquisition of resistance to ADI-PEG20. For an effective metabolic therapy to be developed with ADI-PEG20, a synthetic lethal strategy will need to be identified that will be effective before resistance can occur.

Therefore, we profiled the metabolic alterations that occur in ASS1 deficient sarcoma cells upon arginine deprivation. An unbiased metabolic analysis identified numerous potential metabolic adaptations to arginine deprivation that, when targeted, would shift the cytostatic response to a cytotoxic synthetic lethal response. Further metabolic profiling with stable isotope tracing of U-13C glucose and U-13C glutamine gave a comprehensive summary of the alterations in metabolic phenotype that occur upon arginine deprivation of ASS1 deficient tumors.



With the characterization of the metabolic profile of ASS1 deficient sarcomas before and after arginine deprivation, as well as long term arginine deprivation conditions, we were able to identify a dramatic shift in glucose metabolism and the fate of glucose derived carbons. While cancer cells typically ferment glucose into lactic acid with minor amounts being diverted for

biomass production and arginine deprivation resulted in a significant decrease in the metabolism of glucose derived carbon into lactate. Instead, the bulk of glucose derived carbon was shunted into the serine biosynthesis pathway and was detected in both serine and glycine. Inhibition of the rate limiting enzyme of serine biosynthesis, phosphoglycerate dehydrogenase (PHGDH), and inhibition of the folate dependent single carbon transfer for the generation of glycine from serine were shown to synergize with ADI-PEG20 treatment and were capable of inducing a synthetic lethal cell death response.



Analysis of glutamine metabolism showed a significant increase in the amount of glutamine uptake and metabolism through the TCA cycle upon arginine deprivation. Dramatic increases in the levels of aspartate and asparagine biosynthesis from glutamine derived carbons helped identify the upregulation of glutamine metabolism via glutaminase (GLS) and TCA cycle anaplerosis. The arginine deprivation induced decreases in lactic acid fermentation coupled with the increases in glutamine metabolism via the TCA cycle helped to identify the inhibition of the Warburg effect as a key alteration in cellular metabolism upon ADI-PEG20 treatment. Additionally, the combination of glutaminase inhibition and arginine deprivation was capable of inducing a synthetic lethal response both in vitro and in vivo, highlighting the potential of metabolic based synthetic lethal therapy options for the treatment of ASS1 deficient sarcomas. Taken together, metabolomics analyses were instrumental in the characterization of the metabolic response and the identification of serine biosynthesis and glutamine metabolism as targets for the induction of synthetic lethality upon arginine deprivation in ASS1 deficient sarcomas.

Biography:

Jeff Kremer is a PhD candidate in Molecular Cell Biology at Washington University in St. Louis. His thesis work in the laboratory of Dr. Brian Van Tine has focused on cancer metabolism and investigating the response to arginine starvation in ASS1 deficient sarcomas. His recent publication in Cell Reports marks the culmination of his thesis career and he hopes to continue cancer metabolism research after his graduation from Washington University.



Reference:

"Arginine Deprivation Inhibits the Warburg Effect and Upregulates Glutamine Anaplerosis and

HMT Updates

Event Information

AACR Annual Meeting 2017

April 1-5, Walter E. Washington Convention Center, Washington, D.C., USA

HMT will join AACR in support of research in cancer metabolism. Please drop by our booth #3339 to see what is new and share your research with us so we can create the right metabolic profile to meet your needs.

JMSA New York Life Science Forum 2017

April 8, NYU Langone Medical Center, NY, USA

HMT will have booth exhibition in JMSA (Japanese Medical Society of America) Life Science Forum. Please drop by our booth and update about latest metabolomics topics.

Featured articles

CD44 variant 9 expression as a predictor for gastric cancer recurrence: immunohistochemical and metabolomic analysis of surgically resected tissues

Yamakawa Y., *et al.*, *Biomedical Research (Tokyo)*, **38**, pp. 41-52.

CD44 variant 9 and the heavy chain of 4F2 cell-surface antigen is considered to have regulatory role for regulation of reactive oxygen species, and therefore used as markers of gastric cancer recurrence. The authors applied metabolomics approach to identify the biological mechanism of this marker by using tumor tissue from 103 patients. Various tumor characteristics were significantly associated with CD44v9 expression, including five-year recurrence-free survival rate, but not for CD98hc expression. From the metabolomics profiling, the authors confirmed increase in reduced/oxidized glutathione ratio (GSH/GSSG) in CD44v9-positive tumors, suggesting that CD44v9 is considered to maintain GSH based antioxidant property via the enhanced flux in pentose phosphate pathway.

Titin-truncating variants affect heart function in disease cohorts and the general population

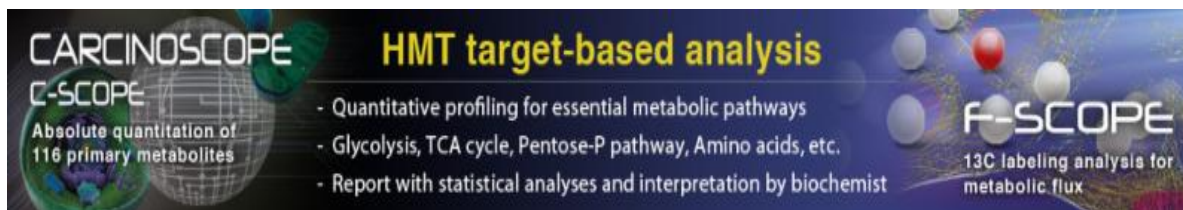
Schafer S., *et al.*, *Nat. Genet.*, **49**, pp. 46-53.

The authors investigated the allelic series of Titin-truncating variants (TTNtv), which commonly cause dilated cardiomyopathy and are encountered in ~1% of the general population. The ribosomal profiling in rat showed the translational footprint of premature stop codons in Ttn and a signature of perturbed cardiac metabolism. From the metabolic profiling of model rat heart tissue, the authors identified the elevation of myocardial substrates, such as BCAA and Glycolytic intermediates, which is known as the feature failing heart and the pressure-loaded non-failing heart. The observed phenomenon across species, and propose molecular and physiological effects on the heart failure.

Metabolite profiling of infection-associated metabolic markers of onchocerciasis

Bennuru S., *et al.*, *Mol. Biochem. Parasitol.*, *in press*.

For the identification of biomarkers supporting the elimination of onchocerciasis, the authors selected non-targeted CE-TOFMS metabolomics which can provide new aspect from previously used methodology of LC-MS platform. From the analysis of patient blood, they screened 286 known metabolites and about 100 putative metabolites based on metabolite databases. For the patient group, the levels of serotonin, hypoxanthine, pipecolic acid and inosine were significantly elevated, whereas the levels of glycerophosphocholine, choline and adenine were significantly lower.



The banner features three main sections: 'CARCINOSCOPE C-SCOPE' with the text 'Absolute quantitation of 116 primary metabolites'; 'HMT target-based analysis' with a bulleted list: '- Quantitative profiling for essential metabolic pathways', '- Glycolysis, TCA cycle, Pentose-P pathway, Amino acids, etc.', and '- Report with statistical analyses and interpretation by biochemist'; and 'F-SCOPE' with the text '13C labeling analysis for metabolic flux'. The background includes molecular models and a globe.

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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