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An Innovation Solution of Multi-Methods Analysis for Central Carbon Metabolism and Broad Coverage of Metabolites Classes

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Introduction

Metabolomics is a powerful technique for understanding biological systems by measuring the abundance of metabolites, however, data interpretation is often complicated by a lack of dynamic information. Similarity, significant changes in targeted metabolomics and fluxomics through a pathway may not result in altered abundance of metabolite intermediates.

To solve these challenges, a novel, innovative targeted metabolomics strategy combining multi-methods was developed to improve the analytical performance by considering peak shape, separation and metabolite coverage of central carbon metabolism, energy metabolism, nucleic acid metabolism and amino acid to cover over hundreds of metabolites in body fluids, tissue or cultured cell line of biological origin. (Figure 1) The new solution was applied to analyze differences biological samples, like the results of bone marrow-derived macrophages (BMDMs) or the mouse models of liver cancer cells with gene knockout showed in this study.

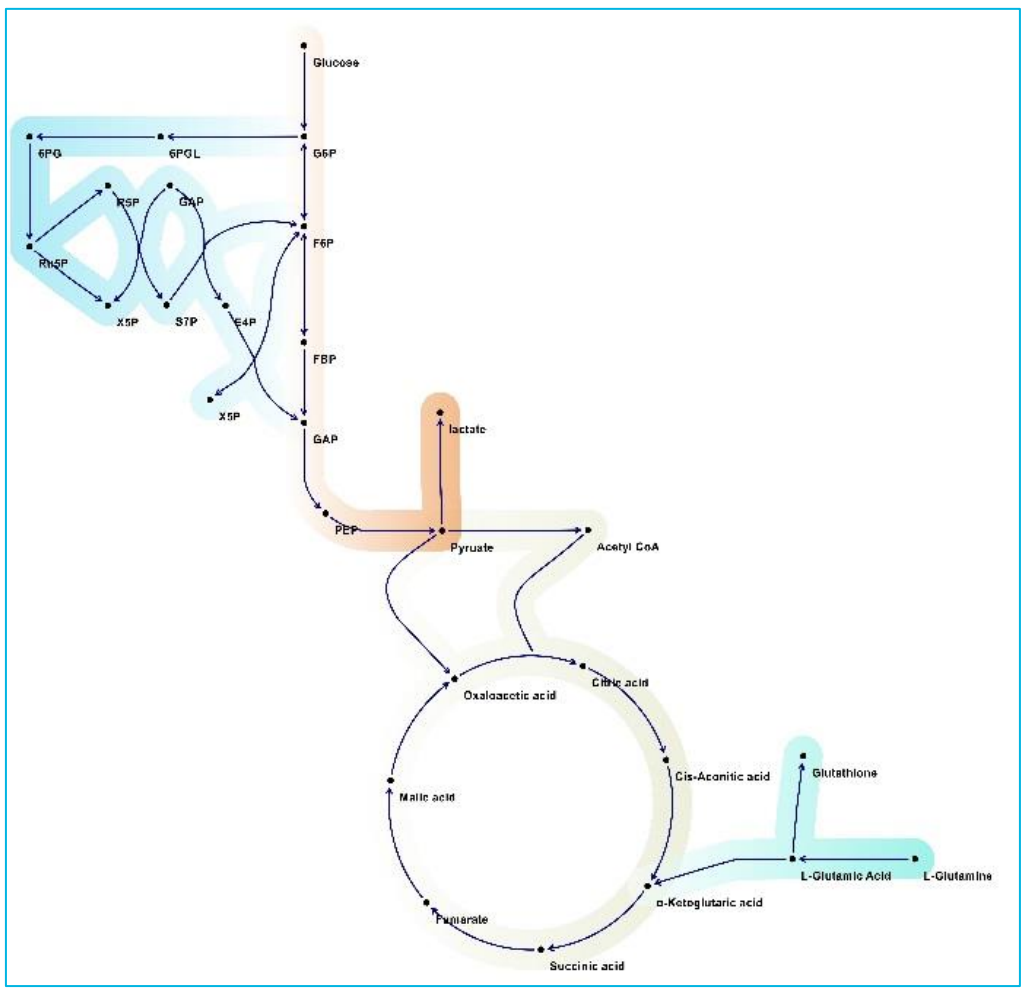


Figure 1. The central carbon metabolism of glycolysis, pentose phosphate pathway, TCA cycle, glutamine metabolism, energy metabolism and Amino acids. (The part of metabolites was not showed.)

Experimental

Normal, reverse and specific phase columns were used for the high coverage metabolites in central carbon metabolism with the difference composition of aqueous and organic solvent with high/low pH as mobile phase. (Table 1)

1290 UHPLC Methods	Column type	Targeted metabolites
1	Amide	100+ (EMP, NTP, NS & AA)
2	C18 (Derivatization)	9 (TCA cycle)
3	Ion-exchange (ROA)	1 (OAA)
4	Mix-Mode	2 (Isocitric/Citric)

Table 1. The multi-methods have high coverage and good separation of central carbo metabolism. (Method 2,3 &4 were not showed the detail information.)

1290 Infinity II UHPLC Method 1

Column: Amide, 2.1X100mm, 1.7µm.

Mobile phase A (MPA): 15mM NH_4CO_2H and 0.3% NH_4OH in water

Mobile phase B (MPB): 15mM NH_4CO_2H and 0.3% NH_4OH in 90% acetonitrile (v/v) aqueous

Column Oven: 40 °C

The linear gradient was used with flow rate, 0.3 mL min^{-1} and the total run time was 20min.

6545 LC/Q-TOF System

Ion source: Dual-AJS

Nebulizer gas: 45psi

Dry gas: 8 L min^{-1}

Dry gas Temperature: 280°C

Sheath gas: 10 L min^{-1}

Sheath gas Temperature: 325°C

Nozzle voltage: 250V (Positive); 1000V (Negative)

Capillary voltage: 3500V (Positive & Negative)

Fragmentor Voltage: 140V

Scan Mass Range: 50-1200 *m/z*

Scan Speed: 3 spectra/sec

Resolution setting: 2GHz (Extend dynamic range mode)

Results and Discussion

In this study, the method 1 cover over hundreds of metabolites. A significant group of polar compounds were not easy to retained, poor peak shapes or undetected with inappropriate or incomplete elution. The easy, quick and convenient solution was to add 5mM of ammonium phosphate (AP) into sample solution and improved the separation chromatography and peak shape, especially sugar phosphate metabolites and related energy metabolism. (Figure 2)

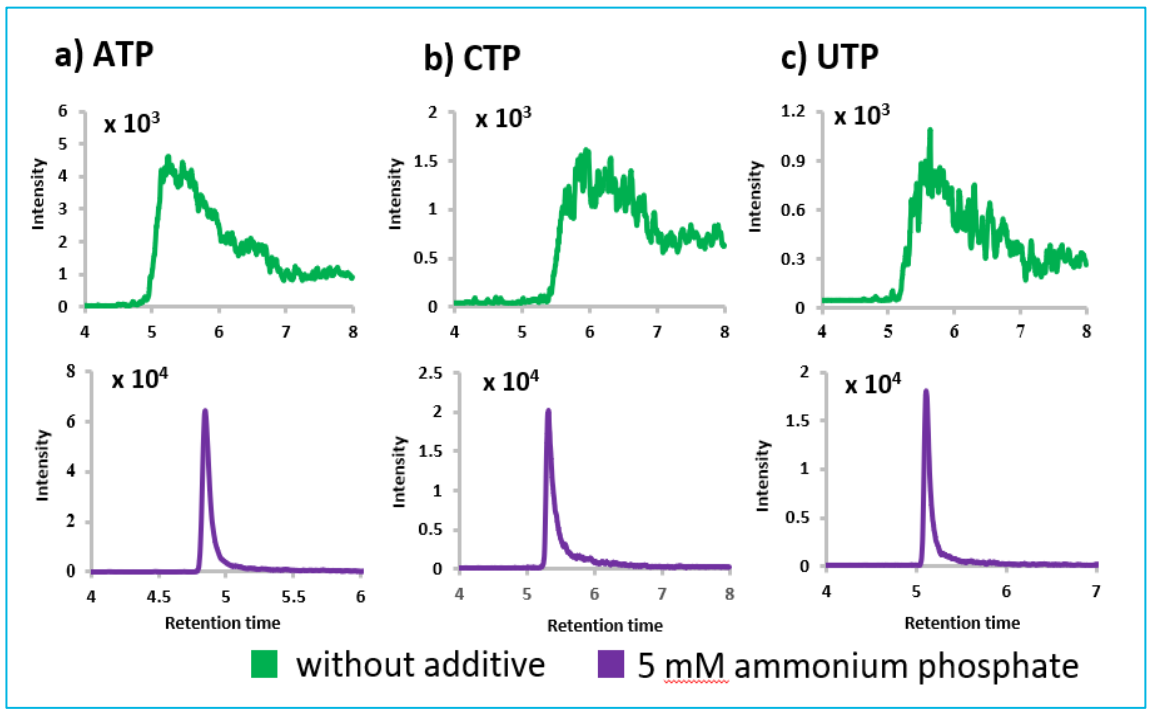


Figure 2. The chromatographic peak shape of triphosphate nucleotide could be substantially improved by the addition of 5mM Ammonium phosphate (top/green vs bottom/purple)

The other challenge was the tricarboxylic acid (TCA) cycle is an interface among central carbo metabolism. Some metabolites are difficult to analyze with method 1 because the isomeric compound separation or poor sensitivity from various samples matrix, especially, α-ketoglutarate (α-KG), Oxaloacetate (OAA) or isocitrate/Citrate. The solutions were developed with O-benzylhydroxylamine (O-BHA) derivatization and special columns in method 2,3 and 4. A rapid, sensitive and reproducible multi-methods was used to assess the trend change of metabolites in cancer cells or disease.

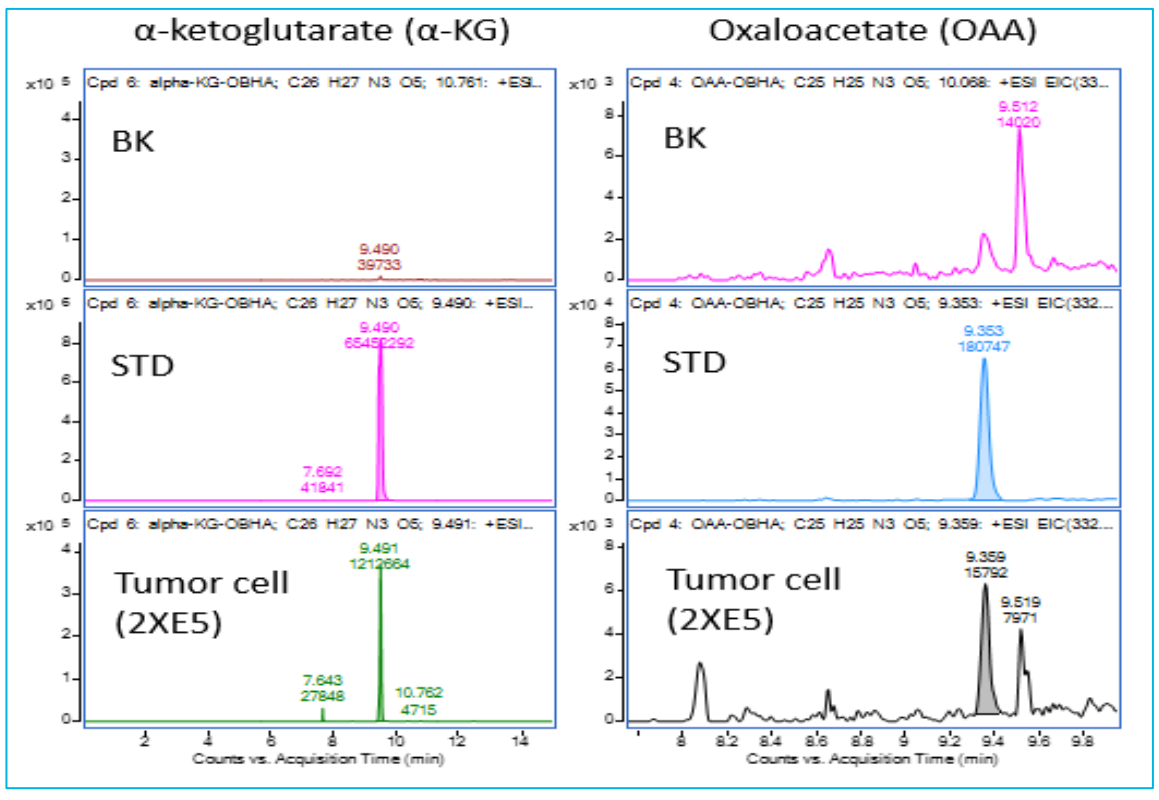


Figure 3. The trace level of α-KG and OAA can be detected with O-BHA derivatization method.

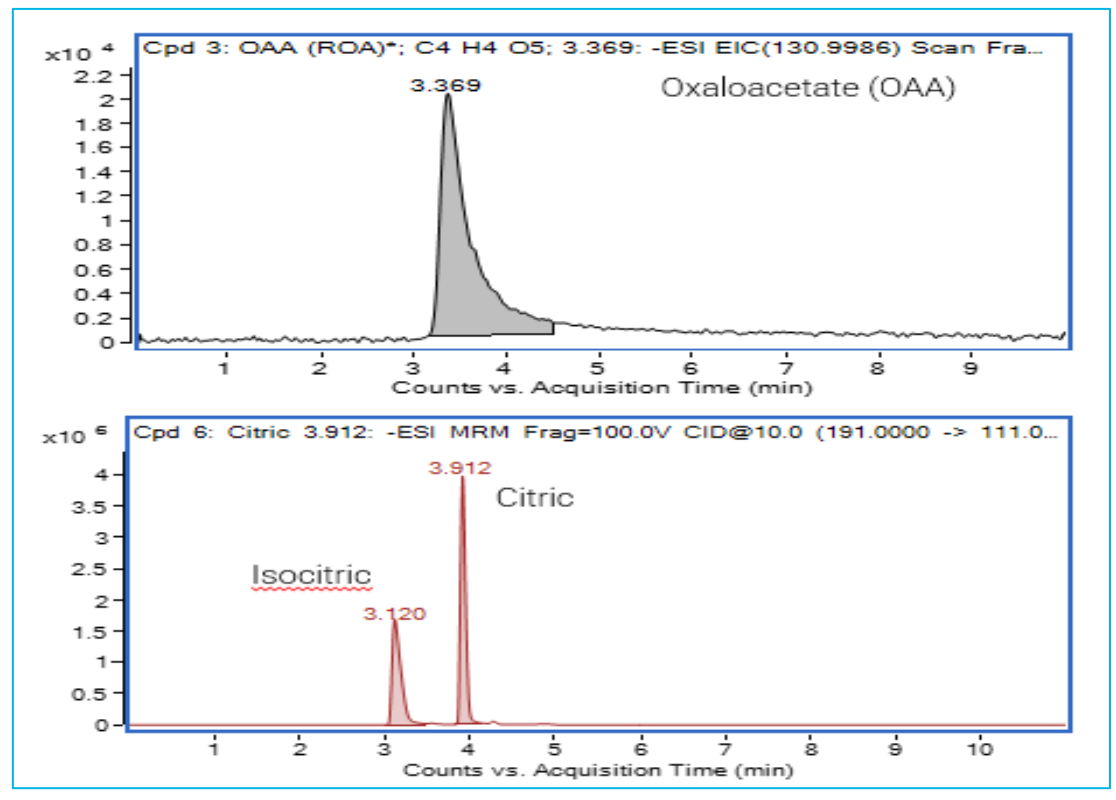
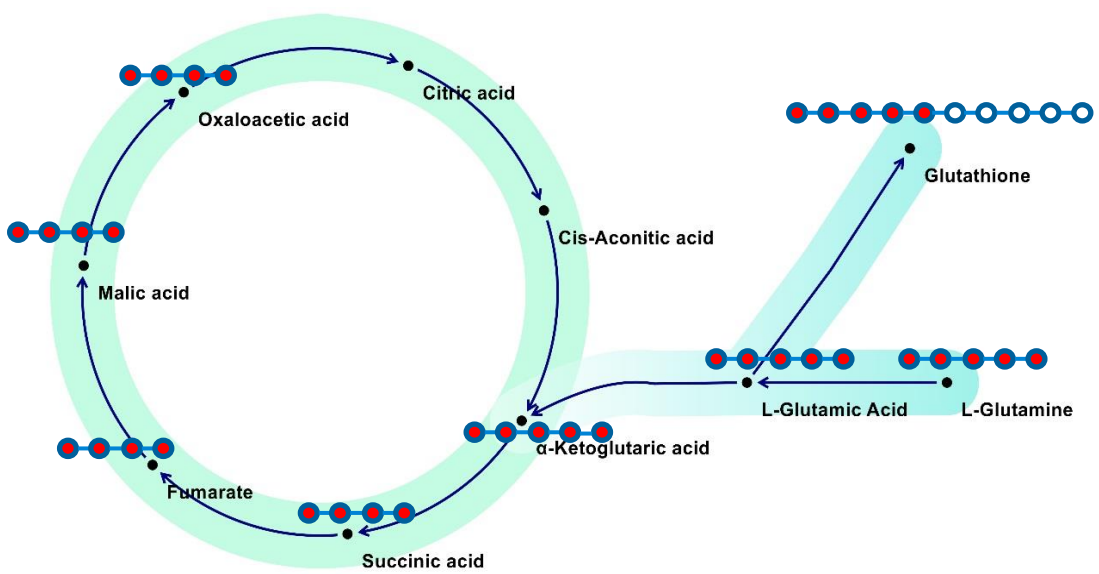


Figure 4. Multi-methods analysis of OAA and Isocitric/Citric with ROA and Mix-Mode column, respectively.

Results and Discussion

To further reveal metabolic reprogramming of glutaminolysis, glutathione biosynthesis and TCA cycle. G gene KO rather than S gene KO upregulates both glycolysis and TCA cycle derived from glucose. (Figure 5)



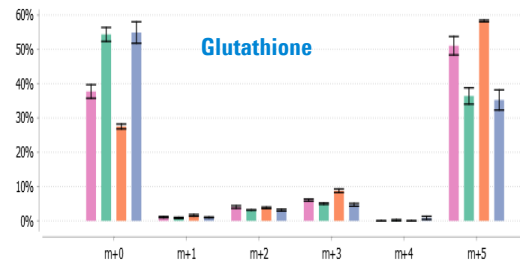


Figure 5. Isotopologues of the intermediate metabolites of glutaminolysis derived from [U-13C5]-glutamine by LC/Q-TOF analysis.

The murine bone marrow-derived macrophages (BMDMs) were treated and pulsed with $^{13}C_6$-Glucose to follow the metabolic reprogramming. (Figure 6)

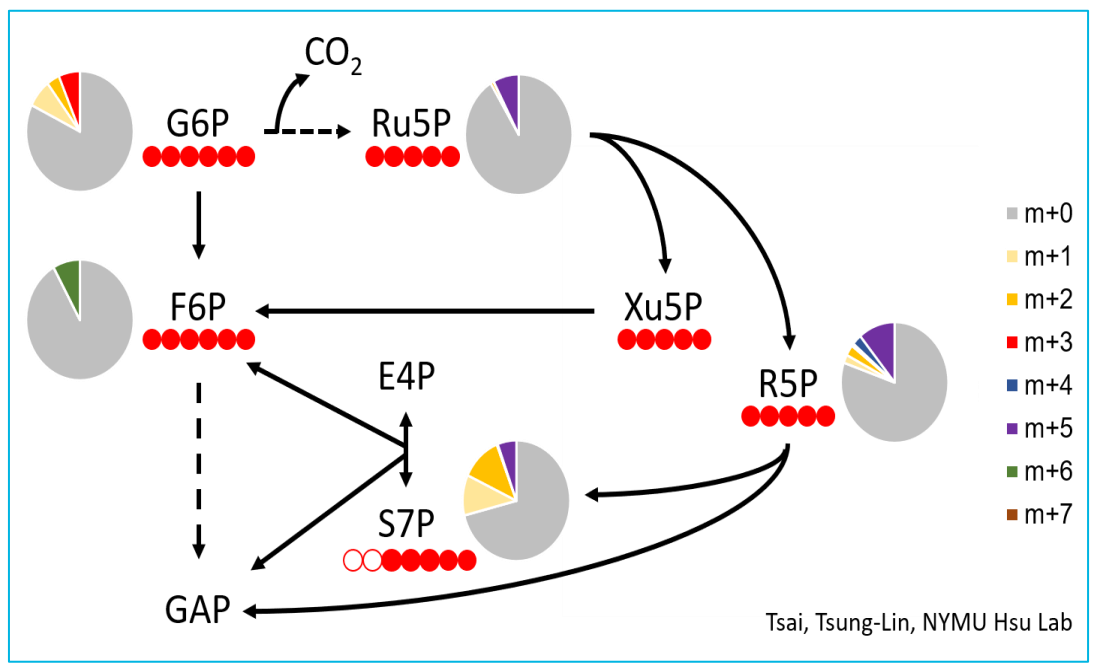


Figure 6. The result of flux analysis showed that the treatment led to a preferential usage of PPP in BMDMs.

Conclusions

The initial results have showed the benefits for the increase sensitivity and separation for the low-level metabolites when adding the AP modifier to sample with best chromatographic peak shape routinely obtained for sugar phosphate metabolites. The potential solution of multi-methods for analyzing the central carbon metabolism and related metabolites in a wide range of biological matrices will be of importance for in vitro, ex vivo and in vivo studies.

References

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