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Isotope Tracing Reveals Metabolic Reprogramming in Cells with UHPLC/Q-TOF and GCMS

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Introduction

Mass spectrometry-based isotope tracing analysis

Metabolic pathways can be impacted to drive energy and biomass production supporting cancer growth. For a better understanding of altered metabolic pathways in cancer cells, we developed a metabolomics approach in combination with stable isotope labeling technique. The isotopic atom (13C, 15N, 18O and 2H, etc) of the tracer molecule can be utilized to reveal the metabolic reprogramming in cancer cells upon genetic modifications and environmental impacts. Mass spectrometry is a powerful tool in measurement of isotopologues of the metabolites labeling with stable isotopes. [1]

In this project, we generated the mouse models of liver cancer: wild type (WT), S gene knockout (S-KO), G gene knockout (G-KO) and double knockout (SG-DKO). The metabolic reprogramming regulated by gene knockout prompted us to ask whether and how S and G gene knockout reprogram the metabolic pathways in cells for rapid proliferation and cancer initiation.

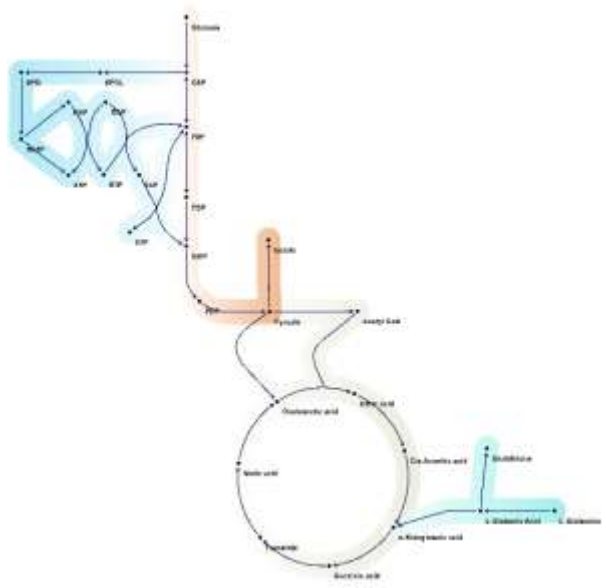


Figure 1. The metabolic pathways of glycolysis, pentose phosphate pathway, TCA cycle and glutamine metabolism.

Experimental

Experiment 1: Agilent GC-MS for analysis using [U-$^{13}C_6$]-glucose as the tracer.

The spectrum of the mass fragmentation in GC-MS can reveal the isotopic distribution of the derivative metabolites. By analyzing the intermediate metabolites in glycolysis and TCA cycle, the isotopomer spectral analysis could reveal metabolic activities of the enzymes like LDH, PDH and PC. Analytes were derivatization with tBDMS, and separation of metabolites occurred on a fused capillary column. The tracer [U-$^{13}C_6$]-glucose was used in cell culture, cells were quenched after 6-h culture with 80% methanol in water. The supernant was dried and then derivatized with tBDMS.

Experiment 2: Agilent UHPLC/Q-TOF MS for analysis using [U-$^{13}C_5$]-glutamine as the tracer.

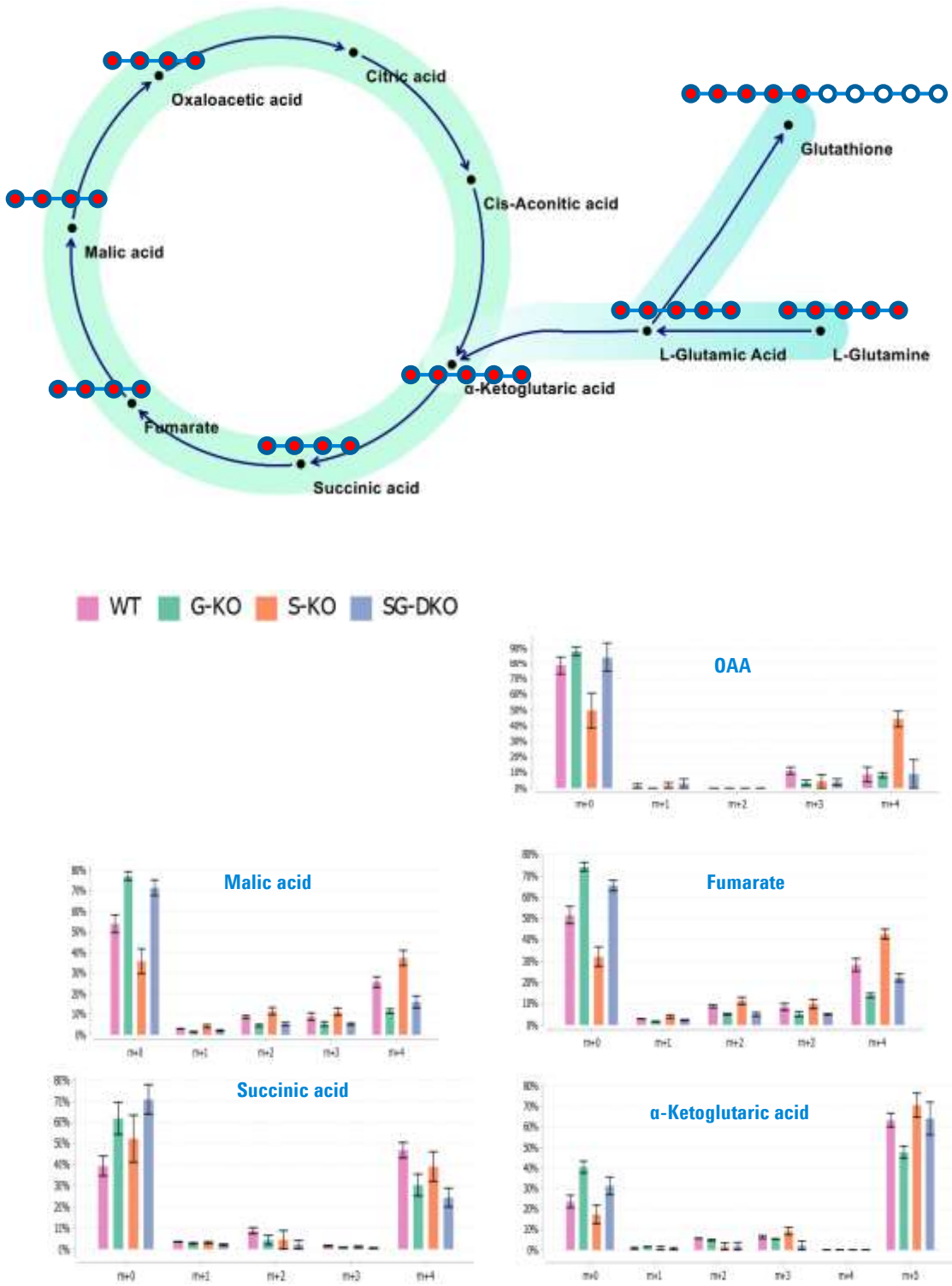
To further reveal metabolic reprogramming of glutaminolysis, glutathione biosynthesis and TCA cycle, we utilized UHPLC coupled to an Agilent 6545 LC/Q-TOF for the analysis of the metabolome on a BEH Amide column. The mobile phase is composed of acetonitrile/water (90:10, v/v) and water containing 15 mM ammonium acetate and 0.1% ammonia hydrate. MassHunter VistaFlux was used for data analysis of the stable labelled experiment.

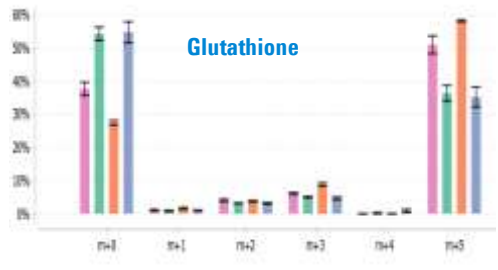
Experiment 3: Agilent UHPLC/Q-TOF MS analysis using [1,2-$^{13}C_2$]-glucose as the tracer.

Regarding the oxidative and non-oxidative pentose phosphate pathway, we used [1,2-$^{13}C_2$]-glucose as the tracer and the same chromatographic condition with experiment 2. VistaFlux was again used for data analysis for tracing stable label incorporation into the pathway.

Results and Discussion

G gene KO rather than S gene KO upregulates both glycolysis and TCA cycle derived from glucose.





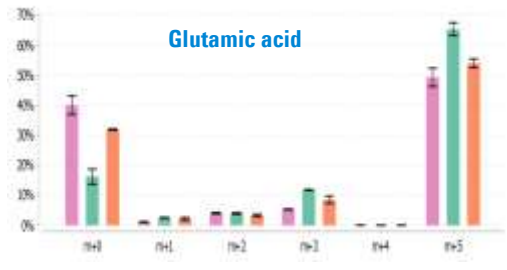


Figure 3. Isotopologues of the intermediate metabolites of glutaminolysis derived from [U-13C5]-glutamine by LC/Q-TOF analysis.

S gene knockout upregulates glutaminolysis .

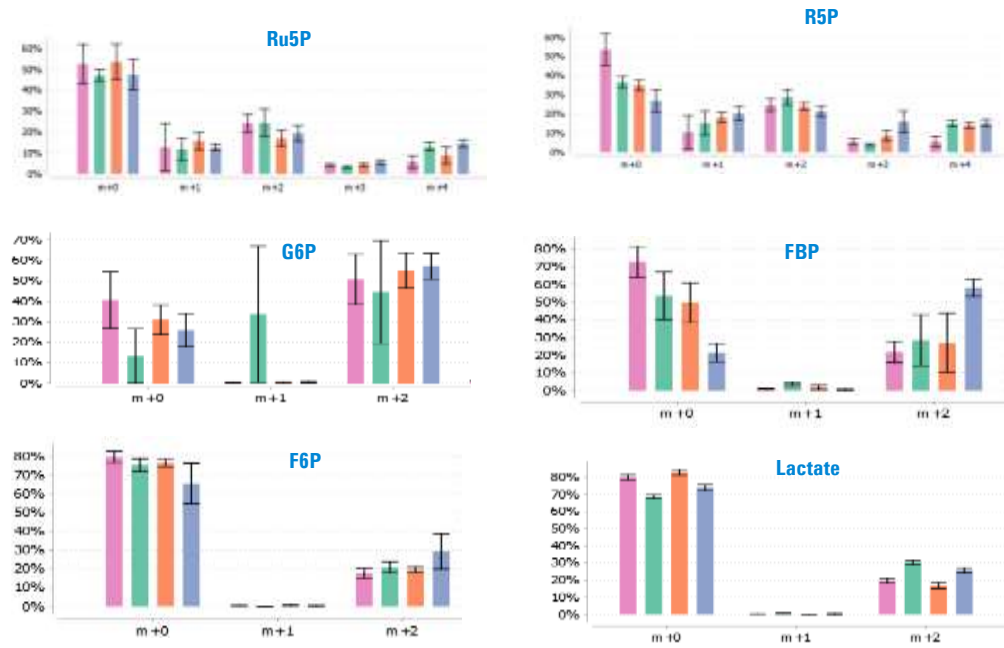


Figure 4. Isotopologues of the intermediate metabolites of glycolysis, oxidative and non-oxidative phases of pentose phosphate pathway derived from [1,2-13C2]-glucose by LC/Q-TOF analysis.

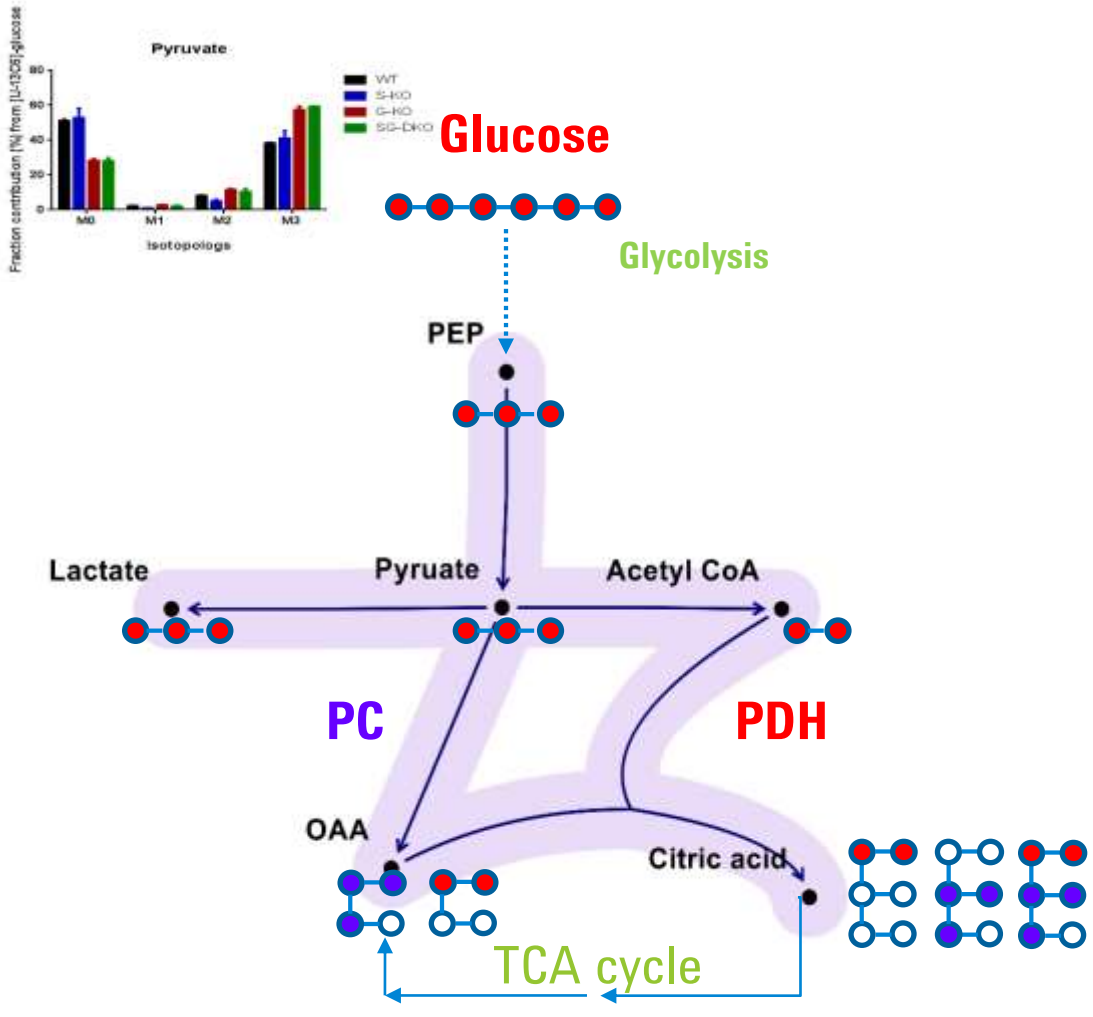
Results and Discussion

Little lactate was generated from oxidative phase of pentose phosphate pathway.

Lactate is mainly produced from glycolysis. Due to the carbon dioxide produced from oxidative phase of pentose phosphate pathway, it would be only one carbon-13 via oxidative arm incorporated in the lactate molecule. We found that very little isotopologue M1 of lactate was detected.

Both knockout genes can upregulates non-oxidative pentose phosphate pathway and reverse glycolysis.

Of great interest, we also found that the isotopologue M4 of ribose 5-phosphate were measured as significantly increased signals in G or S gene knockout or double knockout, indicating that both gluconeogenesis and non-oxidative PPP were upregulated. Meanwhile, double knockout exerts significantly upregulated isotopologue M3 of ribose 5-phosphate, suggested that double knockout promotes oxidative phase of PPP compared to single knockout in the cells.



Conclusions

1) Different tracers in cell culture can reveal the metabolic reprogramming in cells upon gene modifications.
2) The non-oxidative PPP is upregulated induced by gene knockout to support cancer initiation and progression.
3) The isotopic fidelity of Agilent 6545 allows for accurate tracing of stable labels through pathways.
4) MassHunter VistaFlux allows for easy processing of qualitative metabolic flux experiments

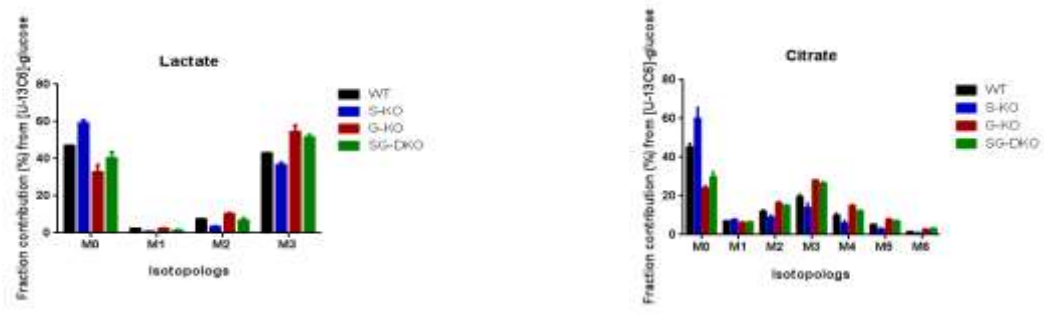


Figure 2. Isotopologues of pyruvate, lactate and citrate derived from [U-13C6]-glucose by GC-MS analysis.

References

1 Krushna, C.P. and Nissin, H. (2014) The Pentose phosphate pathway and cancer. *Trends in Biochemical Sciences* Vol 39, No. 8

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