Differential use of lactate for mitochondria respiration by NSCLC cells

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Introduction

- Identification of metabolic liabilities in cancer cells through metabolic phenotypic screening provides opportunity for novel therapeutic approaches.
- Non-small cell lung cancer (NSCLC) is typically driven by oncogenic mutations in either KRAS or EGFR. However, the impact of these mutations on cellular metabolic phenotype is not well-studied.
- There is emerging evidence to suggest that lactate is a major fuel for cancer cell energy metabolism, especially in the glucose-limited tumor microenvironment.
- In this study, we evaluated metabolic phenotypes of KRAS- and EGFR-mutated NSCLC cells and the contribution of lactate in their bioenergetic metabolism by measuring the real-time ATP production rates and stable isotope incorporation in the TCA cycle using the Agilent Seahorse XF Analyzer and the Agilent LC/Q-TOF Technology respectively.

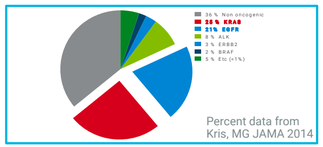

Figure 1: Frequency of oncogenic drivers in adenocarcinoma of the lung detected by the Lung Cancer Mutation Consortium.

Experimental

The Agilent Seahorse XF Real-Time ATP Rate Assay was used to simultaneously measure the two main bioenergetic pathways

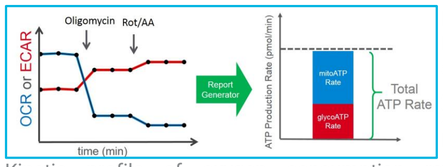

Figure 2. Representative scheme of Agilent Seahorse XF Real-time ATP Rate Assay.

Kinetic profile of oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) measurement. The injection of oligomycin and rotenone plus antimycin A enables calculation of mitochondrial and glycolytic ATP production (Romero, et. al., Agilent White Paper, 2018).

Stable isotope labeling using U-13C lactate: Cells were incubated with 5 mM glucose, 10 mM $^{13}C_3$-lactate +/- MPC or MCT inhibitors for 2hr at 37°C. Samples were snap frozen, extracted with ice cold 8:1:1 methanol: water:chloroform and supernatant was injected after centrifugation. Metabolite samples were analyzed using a 6546 LC/Q TOF coupled to an Agilent 1290 Infinity II LC. Chromatographic separation was performed on an Agilent InfinityLab Poroshell 120 HILIC-Z, 2.1 × 100 mm, 2.7 µm with a HILIC-Z UHPLC Guard column. Data were acquired and analyzed using MassHunter Acquisition software and Agilent Vistaflux.

Results and Discussion

EGFR- and KRAS-mutated NSCLC cells show distinct bioenergetic phenotypes

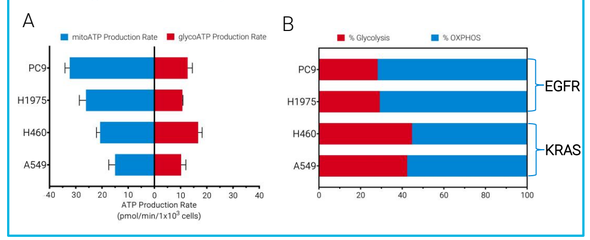

Figure 3: (A) Metabolic Profile of 4 cellular models of NSCLC in standard assay medium (XF RPMI containing 10 mM Glucose, 1 mM Pyruvate, 2 mM Glutamine, pH 7.4). (B) Percent of total ATP production rate from glycolysis and oxidative phosphorylation.

Mitochondrial fuel utilization varies between EGFR- and KRAS-mutated NSCLC cells

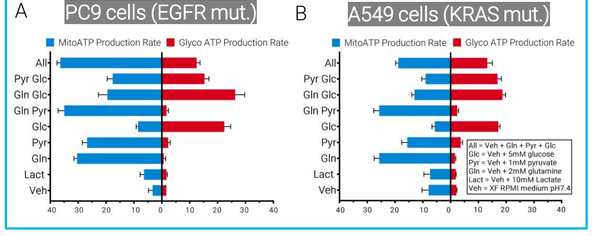

Figure 4: Metabolic profile of ATP production rate in the presence of multiple fuels. (A) PC9 cells can utilize lactate as mitochondrial fuel. (B) A549 cells do not use lactate.

EGFR-mutated NSCLC cells utilize lactate as mitochondrial fuel regardless of glucose availability

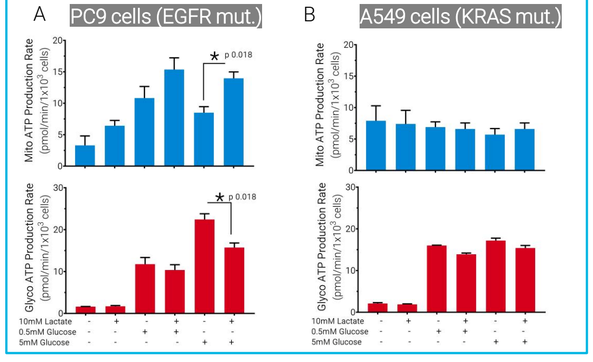

Figure 5: Profile of ATP production rate from glycolysis and mitochondrial respiration +/- lactate and different glucose concentrations in (A) PC9 cells (B) A549 cells.

Results and Discussion

Inhibition of MPC, but not MCT1 suppresses mitochondrial use of lactate in PC9

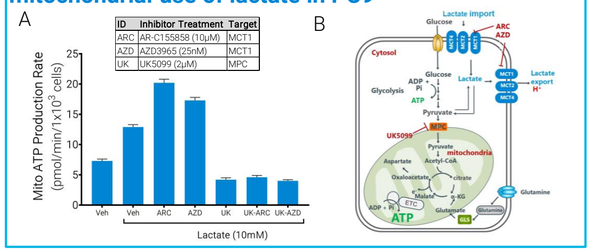

Figure 6: (A) Mitochondrial ATP production rates in PC9 cells treated with MPC inhibitor, UK5099 (UK) or with MCT1 inhibitor, AZD3965 (AZD) or AR-C155858 (ARC) for 30 min prior to addition of 10 mM lactate in the presence of 5mM glucose. (B) Scheme of selected lactate metabolic reactions.

Metabolite tracing data confirms lactate uptake into the cell and incorporation in TCA cycle intermediates mainly thru MPC

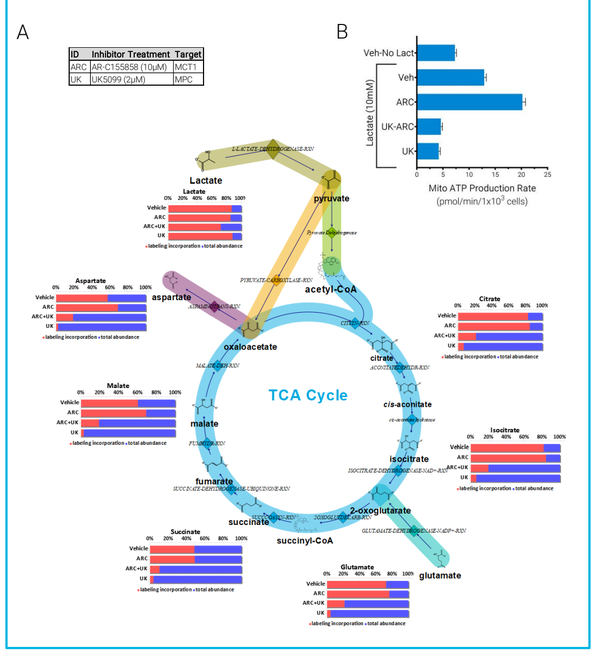

Figure 7: (A) Stable isotope labeling of TCA cycle metabolites using $^{13}C_3$-lactate in PC9 cells (n=3 replicates). (B) Subset of the XF mitoATP production rate which correlates to the metabolic flux data.

Conclusions

- We observed that the two KRAS-mutated NSCLC cell lines (A549 and H460) are relatively more reliant on glycolysis for ATP production than EGFR-mutated cell lines (H1975 and PC9).
- Recent reports strongly suggest that some cancer cells preferentially use lactate as a mitochondrial fuel source. We identified that EGFR-mutated PC9 cells but not A549 cells (KRAS mutant) use lactate to increase mitochondrial ATP production regardless of glucose availability.
- PC9 cells labeled with $^{13}C_3$-lactate showed the incorporation of lactate carbons into TCA cycle metabolites that was blocked in the presence of the MPC inhibitor UK5099. These results correlate with the observed increase in mitochondrial ATP production rate in the presence of lactate using the XF ATP Rate Assay, which was inhibited by pretreatment with UK5099.
- MCT1 inhibition did not block lactate uptake, although MCT1 is known to be used both for import and export of lactate. The presence of other MCT isoform was not excluded and further studies are required.
- These results suggest NSCLC variants have adopted different metabolic phenotypes depending on the oncogenic background. This variation in the bioenergetic phenotype can be a critical factor for a cancer therapy design targeting metabolic vulnerability.

References

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