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Characterization of fuel dependencies in multidrug resistant breast cancer cells

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Introduction

- Multidrug resistance (MDR), a common drug resistant mechanism of cancer cells is characterized by increased expression of ATP-dependent drug exporting pumps which remove cytotoxic compounds from the cytosol of resistant cells.
- MCF7 Dox*, an MDR variant of the MCF7 breast cancer cell line has a highly glycolytic phenotype, with an increased glucose uptake/consumption rate *in vitro* and *in vivo* that contributes to maintaining the MDR phenotype[1]. However, recently studies suggest that mitochondrial modulators have a significant role in avoiding development of MDR phenotypes[2].
- We have previously showed using the Seahorse XF Mito Fuel Flex Test that MCF7 and MCF7 Dox cells are both highly dependent on glucose, however the MDR cells show significantly less dependence on glutamine oxidation (Fig. 1)

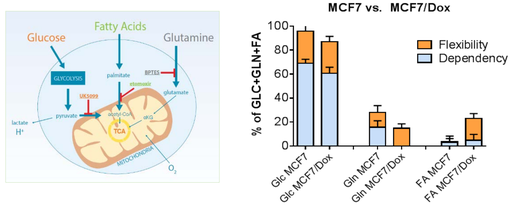


Figure 1: MCF7 Dox loses mitochondrial glutamine dependency. Relative dependency on glutamine oxidation by MCF7 Dox was eliminated, while maintaining a high glucose dependency and capacity when the Mito Fuel Flex test was performed. *MCF7 Dox cells were generously provided by Dr. Robert Gillies at H. Lee Moffitt Cancer Center, Tampa, FL.

Methods

The **Seahorse XF Glycolytic Rate Assay** is an accurate and reliable method for measuring glycolysis, providing measurements of glycolytic rates for basal conditions and compensatory glycolysis following mitochondrial inhibition (Fig. 2A). The calculated rates account for contribution of CO_2 to extracellular acidification derived from mitochondrial/TCA cycle activity and are directly comparable to orthogonal lactate accumulation data (Fig. 2B).

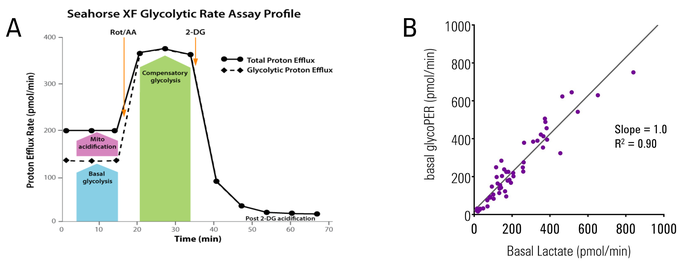
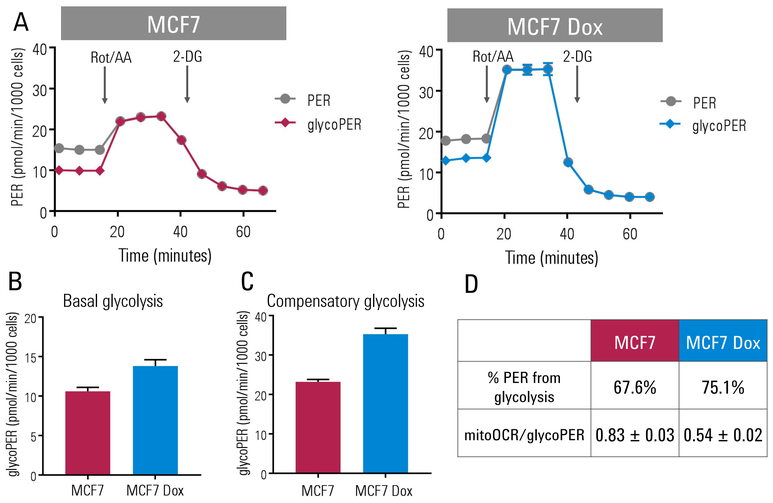


Figure 2. Seahorse XF Glycolytic Rate Assay. (A) Proton efflux from live cells comprises both glycolytic and mitochondrial-derived acidification. Inhibition of mitochondrial function by injecting rotenone & antimycin A (Rot/AA) enables calculation of mitochondrial-associated acidification. Subtraction of mitochondrial acidification from Total Proton Efflux Rate results in Glycolytic Proton Efflux Rate. (B) Glycolytic Proton Efflux rate obtained using the Glycolytic Rate Assay shows tight correlation with basal lactate measured as total lactate accumulated (nmol) in extracellular media during 1 hr (using a fluorescence based lactate kit) and converted to rate values (pmol/min). Results correspond to pooled rate data obtained using the following cell lines (all in triplicate): A431, A549, BAEC, C2C12, CD4+ T cells, H9C2, HepG2, MDA-MB-231, neonatal rat ventricular myocytes (NRVM) and rat cortical neurons and in the absence or presence of the mitochondrial pyruvate uptake inhibitor UK5099.

Results



	MCF7	MCF7 Dox
% PER from glycolysis	67.6%	75.1%
mitoOCR/glycoPER	0.83 ± 0.03	0.54 ± 0.02

Figure 3. Seahorse XF Glycolytic Rate assay results obtained in MCF7 and MCF7 Dox cells. MCF7 and MCF7 DOX cells were plated the day before the assay (25K cell/well) in a XF96 Cell Culture Microplate. After 24 hr, cells were assayed for both oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) in DMEM Base Medium without phenol red, supplemented with 10 mM glucose, 2 mM glutamine, 1 mM pyruvate, 5 mM HEPES, pH 7.4. (A) Representative Glycolytic Rate Assay profile obtained for MCF7 or the MDR variant MCF7 Dox. MCF7 DOX showed a higher glycolytic phenotype as indicated by higher Basal Glycolysis (B), higher Compensatory Glycolysis (C) and lower mitoOCR/glycoPER basal ratio (D).

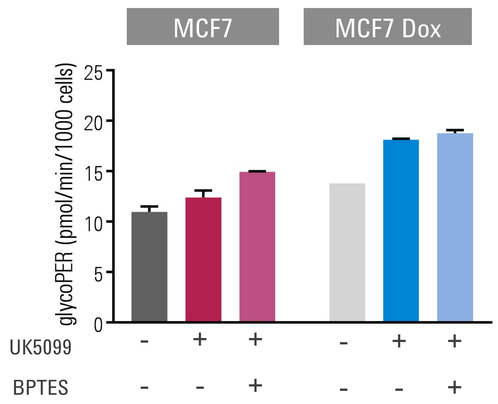


Figure 4. Induced Glycolytic Rates obtained in MCF7 and MCF7 Dox cells with mitochondrial fuel oxidation inhibitors. The Glycolytic Rate Assay was performed in MCF7 and MCF7 Dox cells after injection of the mitochondrial pyruvate carrier (MPC) inhibitor UK5099 (2 µM), and glutaminase I inhibitor BPTES (3 µM). Glycolytic rates increased after inhibition of mitochondrial pyruvate oxidation, in agreement of high glucose dependency obtained using the Mito Fuel Flex Test. Additionally, inhibition of glutamine oxidation has a lower impact in MCF7 Dox compared to MCF7, confirming the lack of dependency of MCF7 Dox on glutamine oxidation. XF assay conditions are the same as described in Fig. 3 above.

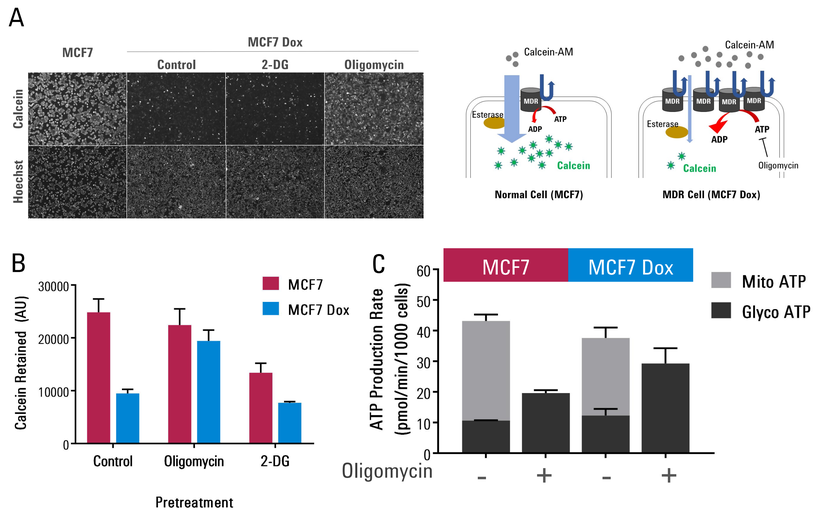


Figure 5. MDR-mediated drug efflux relies on ATP generated by mitochondria. MCF7 and MCF7 Dox were incubated in the presence of calcein-AM in DMEM Base Medium without phenol red, supplemented with 10 mM glucose, 2 mM glutamine, 1 mM pyruvate, 5 mM HEPES, pH 7.4 for 10 min at 37°C with or without pretreatment of 2-deoxy-D-glucose (2-DG, 50 mM) or oligomycin (1 µM) for 10 min. The calcein-AM retained in cells were compared by using the fluorescent images captured (A) and the whole well fluorescent intensities measured (B) by BioTek® Cytation™ 5. (C) Total ATP formation was calculated as the sum of Glycolytic ATP Rate formation (equivalent to glycoPER) and mitochondrial-derived ATP Rate formation that was estimated from the ATP-coupled OCR and assuming a P/O ratio of 2.79 [3].

Conclusions

- Using the Seahorse XF Glycolytic Rate Assay, basal glycolytic rates and compensatory glycolysis (when mitochondrial ATP production is blocked) are higher in MCF7 Dox cells compared to wild type cells, confirming the highly glycolytic phenotype of the MCF7 Dox variant.
- Addition of the MPC inhibitor, UK5099, induced a metabolic switch to glycolysis, confirming the strong dependency and low flexibility of both cell variants for glucose as mitochondrial fuel. In addition, a further glycolytic shift is induced after combined inhibition of glutamine/pyruvate oxidation in MCF7 cells that is not observed in MCF7 Dox cells, in agreement with their lack of dependency for glutamine as a metabolic fuel.
- Calcein-efflux studies strongly suggest that the MDR mechanism relies on mitochondrial generated ATP for maintaining a chemo-resistant phenotype.
- Fuel dependency characterization may have potential therapeutic relevance to understand therapy resistance.

References

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2. Fernandez-Cabezudo, M.J., et al., Deficiency of mitochondrial modulator MCJ promotes chemoresistance in breast cancer. *JCI Insight* 2016, *1*.
3. Mookerjee, S.A., et al., Quantifying Intracellular Rates of Glycolytic and Oxidative ATP Production and Consumption Using Extracellular Flux Measurements. *J Biol Chem* 2017.

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