Measuring the Metabolic Switch in Cancer Cells

Metabolic Profile Provides New Insight into Choices of Therapeutic Intervention

Application Brief

Introduction

In the 1930's, Otto Warburg described how cancer cells use aerobic glycolysis in preference to oxidative phosphorylation[1], a phenomenon now known as the Warburg effect. Warburg further observed that this high rate of aerobic glycolysis provides sufficient ATP for energy stores and the intermediate metabolites needed to support tumor growth. Nonetheless, the reason that cancer cells demonstrate enhanced glycolysis and reduced mitochondrial respiration remains unclear and an active area of research.

Robinson; *et al.*[2] used the Agilent Seahorse XF24 Extracellular Flux Analyzer to demonstrate that the glycolytic phenotype in Z138 cells (Mantle Cell Lymphoma cells) is more complicated than described by Warburg. Moreover, Robinson determined that more subtle changes in metabolic phenotype correlate to the lymphoma cells' sensitivity to apoptosis therapy.

Initially, Robinson inhibited glycolysis by treating the Z138 cells with 2-deoxyglucose (2-DG) to block hexokinase, the first enzyme in the glycolytic pathway, and observed a reverse Warburg change in metabolism as expected. He showed that 2-DG treatment of Z138 cells produced an almost complete inhibition of glycolysis (ECAR), and an approximately 30 % reduction in oxidative phosphorylation (OCR) as shown in Figure 1A.

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Robinson observed a surprisingly different phenotypic change in metabolism when he inhibited glycolysis by growing the Z138 cells in glucose-free medium, as illustrated in Figure 1B. Although depriving the cancer cells of glucose caused a reverse Warburg phenotype, the Z138 cells oxidative phosphorylation levels increased by approximately 60 %, in contrast to the decrease observed with 2-DG. This aerobic phenotype was most notable after 20 hours of growth in glucose-free media. Continued growth under these conditions resulted in a slower growth rate an almost complete conversion to oxidative phosphorylation. Robinson then demonstrated that the 2-DG treated cells, with an enhanced aerobic phenotype, were more sensitive to TNF-related apoptosis-inducing ligand (TRAIL) treatment (data not shown).

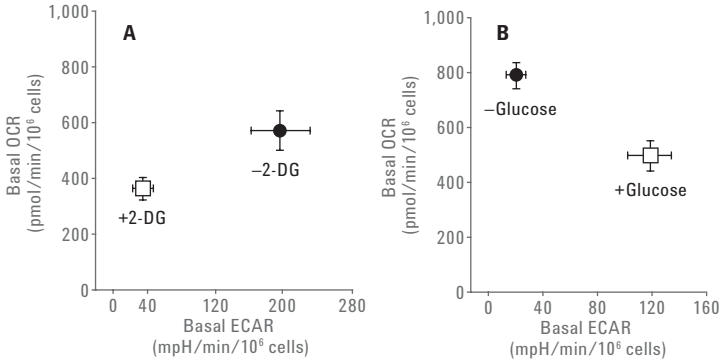

Figure 1. Metabolic Switch induced by 2-DG inhibition of glycolysis results in a less aerobic phenotype than metabolic switch induced by glucose-free culture. A) Z138 cells cultured for 20 hours in 11 mM glucose, 1 mM pyruvate and 2 mM glutamax supplemented medium with (□) or without (■) 5 mM 2-DG. B) Z138 cells cultured for a minimum of 7 days in 2 mM glutamax and 1 mM pyruvate containing media with (□) or without (■) 11 mM glucose.

Results and Discussion

While differentiated, specialized cells typically have a large spare capacity to respond to increased ATP demand, cancer cells often have a low spare capacity. Highly proliferating cells typically have a basal OCR closer to the maximal OCR owing to the highly anabolic nature of these cells. The Agilent Seahorse XF Cell Mito Stress Test Kit identifies this phenotype in cancer cells, and also allows for the determination of the proportion of basal OCR that is linked to the production of ATP.

Many cancer treatments work by inducing apoptosis through an extrinsic pathway. The extrinsic pathway is initiated by the extracellular binding of members of the TNF ligand family, including TNF-related apoptosis-inducing ligand (TRAIL). TRAIL can be selectively toxic to tumor cells, and initiates apoptosis through the death-inducing signaling complex (DISC). ATP is needed for this extrinsic-mediated apoptotic cell death, apoptotic cells have been shown to have elevated ATP levels. Zamaraeva; *et al.*[3] have shown that cells deprived of glucose have decreased ATP levels and are sensitized to death-receptor mediated apoptosis. Conversely, Robinson found that prolonged exposure of Z138 cells to glucose-free media mediated a metabolic switch from aerobic glycolysis to oxidative phosphorylation. Under these conditions, ATP levels were maintained and the cells were significantly less sensitive to TRAIL. By inhibiting aerobic glycolysis with 2-DG treatment, ATP levels were found to be decreased and the Z138 cells showed an increased sensitivity to TRAIL.

Teperino; *et al.*[4] used the Agilent Seahorse XF24 Analyzer to identify another metabolic switch that occurs in muscle and brown fat cells as a consequence of hedgehog signaling. Hedgehog signaling triggers a rapid Warburg-like metabolic reprogramming from oxidative phosphorylation to aerobic glycolysis.

This metabolic reprogramming, mediated by Smo-Ca^{2+}-Ampk signaling, affects robust glucose uptake *in vivo* in mouse and human myocytes. This stimulation was shown to be induced by several hedgehog inhibitors. The analysis, done on the Agilent Seahorse XF24 Analyzer, made use of a Smo signaling surrogate, smoothened agonist (SAG), which caused an increase in ECAR and OCR upon stimulation.

Yoshida; *et al.*[5] reported that the molecular chaperone, TNF receptor-associated protein (TRAP1), regulates the metabolic switch between mitochondrial respiration and aerobic glycolysis. TRAP1 is found predominantly in mitochondria, though its impact on mitochondrial metabolism is unknown. Yoshida used TRAP1-null cells and transient TRAP1 mutants on an Agilent Seahorse XF96 Analyzer to show that TRAP1 regulates a metabolic switch between oxidative phosphorylation and aerobic glycolysis in immortalized mouse fibroblasts and in human tumor cells. TRAP1 deficiency promotes increased mitochondrial respiration, fatty acid oxidation, accumulation of TCA intermediates, ATP, and ROS, while suppressing glucose metabolism.

Materials and Methods

Cells and compounds

Z138 cells were seeded into Agilent Seahorse XF24 Cell Culture Microplates coated with Cell-Tak (Corning) at 4.0 × 10^5 cells/well, and incubated at 37 °C for 1 hour before analysis. FCCP, rotenone, and 2-DG were from Sigma-Aldrich.

XF Analysis

XF analyses were performed in the Agilent Seahorse XF24 Extracellular Flux Analyzer. The Agilent Seahorse XF24 Analyzer creates a transient micro-chamber of only a few microliters in specialized cell culture microplates. This enables the oxygen consumption rate (OCR), and the extracellular acidification rate (ECAR), to be monitored in real time.

To prepare for the assay, illustrated in Figure 2, the Z138 cells were switched from culture medium to assay medium, the sensor cartridge was loaded with experimental compounds, calibrated, and placed over the culture plate containing the prepared Z138 cells. After mixing, OCR and ECAR were measured simultaneously for 3 minutes to establish a baseline rate. Then, 75 µL of testing agent prepared in assay medium were injected into each well to the final working concentration. This was followed by mixing for 5 minutes to expedite compound exposure to cellular proteins, after which OCR and ECAR measurements were made. Three baseline rates and three response rates after compound addition were measured in triplicate and averaged:

- Oligomycin (1 µM), an inhibitor of ATP synthase that allows a measurement of ATP-coupled oxygen consumption through OXPHOS
- Carbonyl cyanide 4-trifluoromethoxyphenylhydrazone (FCCP) (300 nM), an uncoupling agent that allows maximum electron transport and therefore a measure of maximum OXPHOS respiration capacity
- 2-DG (100 mM), an inhibitor of glycolysis
- Rotenone (1 µM), an inhibitor of complex I of the mitochondrial respiratory chain that allows a precise measurement of mitochondrial uncoupling

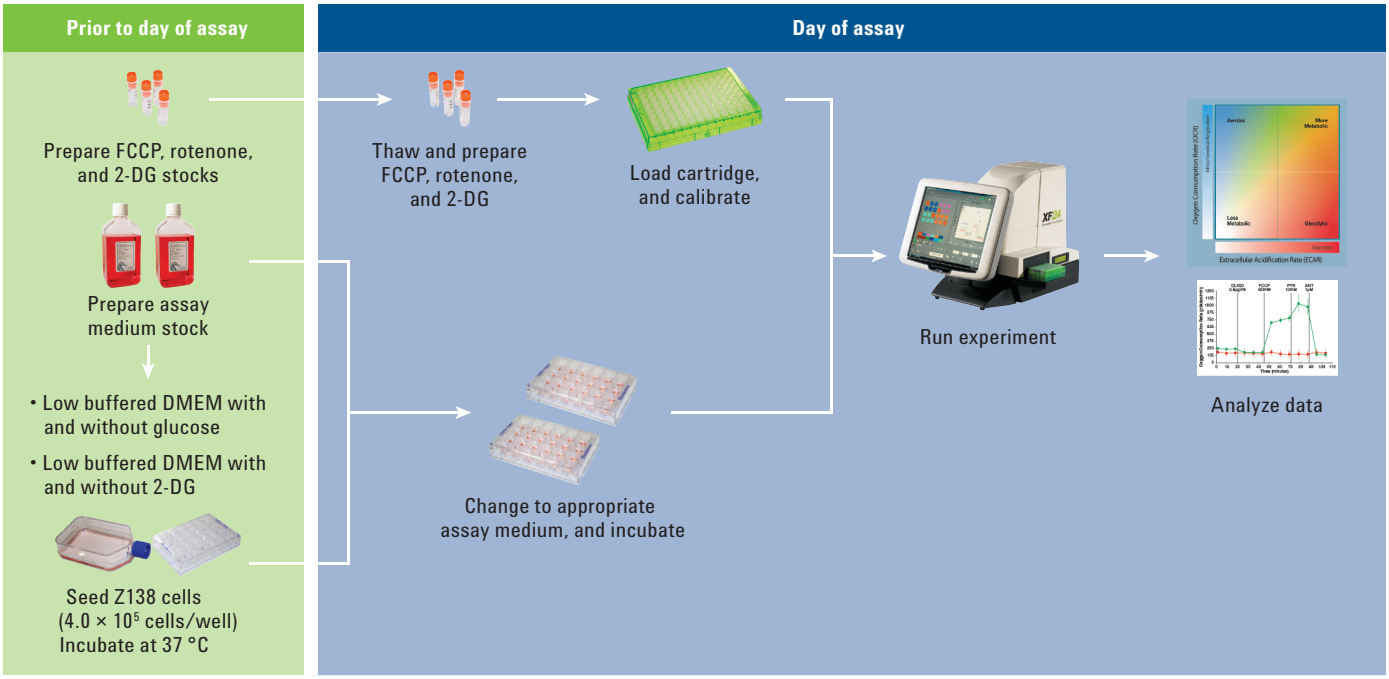

Figure 2. Flow chart of the XF24 assay.

References

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