Measuring Glycolysis and Oxidative Metabolism in Cancer Cells

Real-Time Assay Enables Scientists to Connect Metabolic Pathways With Oncogenic Phenotypes

Application Brief

Introduction

Measuring the metabolic pathways in cancer cells, and their co-dependent or compensatory interplay, is important for understanding transformation mechanisms, mechanisms of malignant growth, cancer cell drug resistance, and for discovering potential drugs that are relatively specific for cancer cells.

While normal cells generate ATP and biosynthetic precursors through a combination of oxidative and glycolytic metabolism, cancer cells dramatically reprogram their metabolism to support rapid, invasive, and metastatic growth. This altered metabolism coincides with oncogene activation or loss of tumor suppressor genes in multiple signaling pathways, and confers competitive advantages to transformed cells[1].

Cells with a glycolytic phenotype exhibit significantly higher rates of proton production (extracellular acidification rate, ECAR) than cells using oxidative phosphorylation (oxygen consumption rate, OCR). Shifts in substrate utilization and metabolism, characteristic of cancer cells, can be detected conveniently and simultaneously through detection of the OCR, to quantify mitochondrial respiration, and the ECAR, an indicator of glycolysis. Such measurements can characterize the metabolic programming of individual cancer cell types, their stage of transformation, and forecast their metastatic potential.

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Wu; *et al.*[2] validated OCR and ECAR as indicators of cellular respiration and glycolysis respectively, by measuring their responses to three well-defined modulators of mitochondrial respiration and glycolysis in the A549 human cancer cell line. 2-4 DNP uncouples respiration from ATP synthesis, stimulating both respiration and glycolysis. 2-DG inhibits hexokinase, the first enzyme required for glycolysis. Rotenone inhibits mitochondrial NADPH dehydrogenase/complex I, specifically inhibiting mitochondrial respiration. OCR was stimulated by 2-4 DNP and inhibited by rotenone, while ECAR was stimulated by 2-4 DNP, and inhibited by 2-DG. This demonstrates that OCR specifically reflects the rate of mitochondrial respiration and ECAR specifically reflects the rate of glycolysis (Figure 1A).

Wu; *et al.*[2] also determined the relative contributions of glycolysis in relation to total extracellular acidification rates in H460 and A549 cells with oxamate, a glycolysis inhibitor that acts by inhibiting lactate dehydrogenase to prevent conversion of pyruvate to lactate. Oxamate-sensitive ECAR reflects glycolytic acidification, and oxamate insensitive ECAR is due to non-glycolytic acidification, mostly due to carbon dioxide. The ECAR of both cell types decreased to 20 % of their respective baselines following exposure to oxamate, indicating that glycolysis accounted for about 80 % of total ECAR in these cell lines. The ECAR of H460 cells was significantly higher than that of A549 cells, suggesting the former is more glycolytic. Both cell lines are known to be metastatic in animal models (Figure 1B).

In summary, the ability to measure mitochondrial respiration and glycolysis simultaneously, and in real time, allows one to determine the response of these two pathways to ATP demand, and, indirectly, biosynthetic needs. In the case of cancer, it is becoming clear that malignancy is inextricably linked to alterations in metabolic programming.

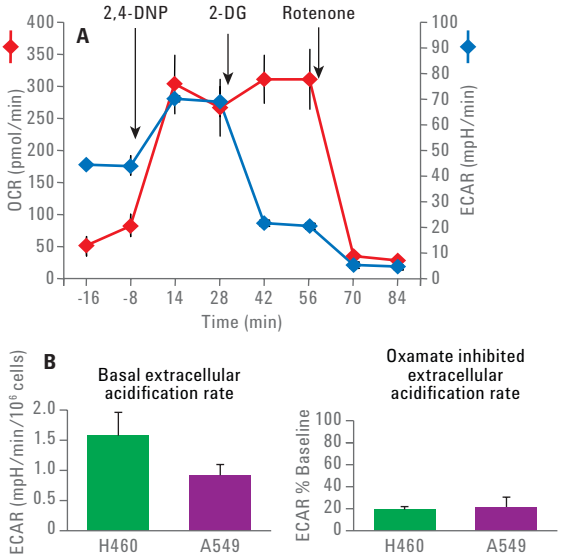

Figure 1. Validation of OCR and ECAR as indicators of cellular respiration and glycolysis. A) The mitochondrial uncoupler, 2-4 DNP, glycolysis inhibitor 2-DG, and mitochondrial complex 1 inhibitor rotenone were injected sequentially into A549 cells. OCR was stimulated by 2-4 DNP and inhibited by rotenone, while ECAR was stimulated by 2-4 DNP and inhibited by 2-DG, validating OCR and ECAR as indicators of cellular respiration and glycolysis, respectively. B) The contribution of glycolysis to total extracellular acidification rates in H460 and A549 cells was determined using oxamate. The ECAR of both cell types decreased to 20 % of their respective baselines following their exposure to oxamate.

Results and Discussion

The Agilent Seahorse XF Analyzer enables characterization of glycolysis and mitochondrial respiration without the need for sequential sampling and spectrophometric lactic acid measurements.

Wu; *et al.*[2] profiled the metabolic phenotype of two human cell lines, H460 and A549. H460 cells were shown to be more glycolytic than A549 cells. The ATP content of both cell types remained unchanged by rotenone (data not shown), suggesting glycolytic ATP synthesis could sustain cellular energy balance. However, the ATP content of H460 and A549 cells were differentially sensitive to 2-4 DNP. A549 cell intracellular ATP content was reduced by 40 % in the presence of 2-4 DNP, compared to the untreated control, while that of H460 cells was not significantly changed.

Delgado; *et al.*[3] used the Agilent Seahorse XF Analyzer to demonstrate that Kaposi's sarcoma-associated herpesvirus (KSHV), the etiological agent of Kaposi's sarcoma, drives infected cells towards increased ECAR and decreased OCR. They concluded that the Warburg effect is necessary for maintaining KSHV latency and adapting the infected cells to tumor microenvironments, allowing Kaposi's sarcoma tumors to seed.

DeGroof; *et al.*[4] analyzed the relationship between metabolism and malignant transformation at different stages of oncogenic progression. Transformation by H-RasV12/EIA caused increases in OCR, accompanied by cell death at low passage number, and increased growth rates and tumor forming potential with high passage number. In the high passage cells, OCR declined while ECAR increased, consistent with the Warburg effect.

Gohil; *et al.*[5] used a nutrient-sensitized screening strategy to identify drugs that shift cellular energy metabolism. Cell cultures using galactose as the sole sugar source forces cells to rely on OXPHOS. By screening a chemical library that included FDA-approved compounds for drugs that selectively inhibit growth and proliferation in galactose-relative-to-glucose media, the investigators identified several compounds that redirected oxidative metabolism to glycolysis, including over-the-counter antiemetic meclizine. The investigators confirmed results of primary viability assays used for initial drug screening by measuring OCR and ECAR. Meclizine reduced OCR in a dose-dependent manner in cells cultured in glucose-rich medium, with a concomitant increase in ECAR. The authors suggested that their screening method might find application in screening large libraries of compounds in the context of cellular energy homeostasis.

Materials and Methods

Cells and compounds

H460 and A 549 cells were maintained in growth medium consisting of RPMI 1640, 10 % FBS, penicillin and streptomycin (Invitrogen) and seeded at 20,000 cells per well into Agilent Seahorse XF24 Cell Culture Microplates. Concentrated stocks of 1,000 mM 2-DG was prepared in assay medium. Concentrated stocks of 2-4 DNP and rotenone were prepared in DMSO. 2-4 DNP was diluted to 10× working concentration in assay medium and adjusted to pH 7.4. Rotenone was diluted to 13× working concentration in assay medium.

XF Analysis

Assays were performed in the Agilent Seahorse XF Extracellular Flux Analyzer, a fully integrated multiwell instrument that measures uptake and excretion of metabolic end products in real time. OCR and ECAR were measured using an Agilent Seahorse XF cartridge. This disposable cartridge contains 24 solid-state, dual-fluorescent biosensors (O_2 and pH). Each sensor is also equipped with four drug injection ports for delivering test agents into wells during an assay. OCR is reported in pmol/min and ECAR in mpH/min.

To prepare the assays as illustrated in Figure 2, the treated and untreated H460 or A549 cells were switched from culture medium to assay medium (low-buffered RPMI 1640 containing 1 mM phosphate). Following baseline measurements, 75 µL of testing agent prepared in assay medium was injected into each well to reach final working concentrations. Following 5 minutes of mixing to expose cells to the test reagents, OCR and ECAR were measured. For time-resolved experiments, multiple compound injections were made at the time points indicated.

Measuring the extracellular acidification rate (ECAR) provides a convenient method for detection of glycolytic flux in cancer cells in response to genetic changes, or to biologically active compounds. The Agilent Seahorse XF Analyzer simultaneously measures OCR and ECAR, providing a more comprehensive assessment of cellular bioenergetics, and analysis of the dynamic interplay between the two major energy-yielding pathways in cancer cells and other cell types.

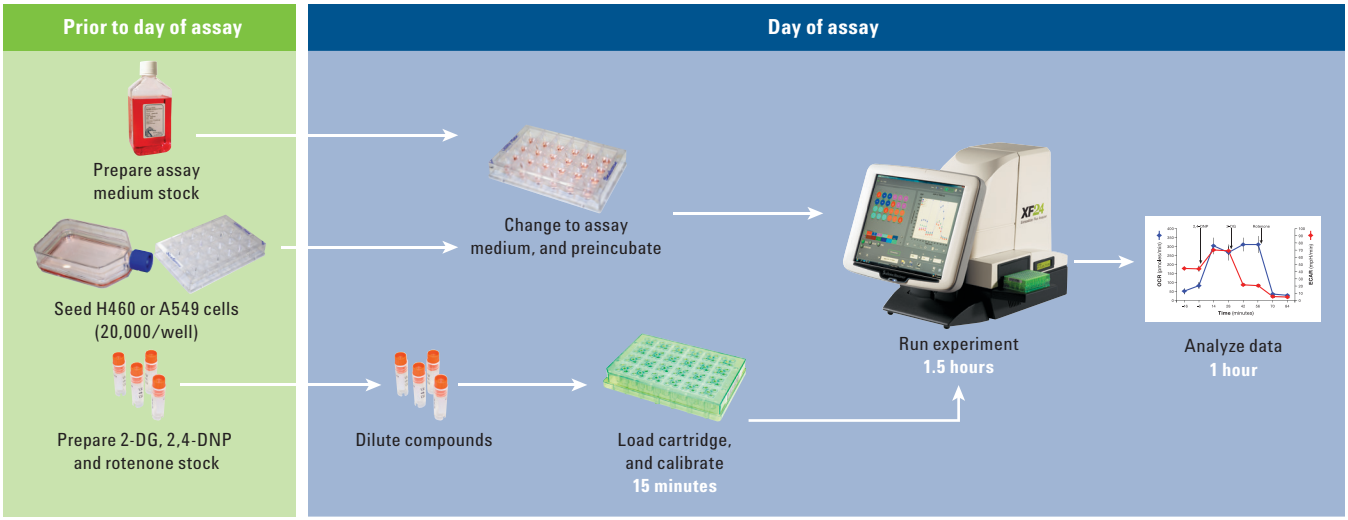

Figure 2. Flow chart of the XF assay.

References

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Additional Reading

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