Application Note
Cancer Research

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Targeting Energy Metabolism for Cancer Therapeutic Discovery using Agilent Seahorse XF Technology

Authors

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Abstract

Altered energy metabolism is now recognized as a driver in many diseases. In cancer research, significant advances have been made in defining druggable targets, with metabolic intermediates emerging as promising therapeutic targets. Agilent Seahorse XF technology provides critical functional measurements in live cells to identify potential druggable gene and protein targets, and to validate their role in cancer cell proliferation, adaptation, and survival. By measuring bioenergetic phenotype, cellular ATP production rate, and mitochondrial and glycolytic function, metabolic vulnerabilities in cancer cells can be revealed to advance cancer therapeutics.

Introduction

Background: Advancing opportunities in cancer drug therapies

Cancer cell proliferation is a dynamic process that demands significant raw materials and energy. Conventional views have commonly inferred that cancer cells rely on upregulated glycolysis for their proliferation requirements, often characterized as the Warburg effect [1, 2]. However, with advancements in metabolic analysis techniques, it is now clear that some cancer cells preferentially utilize mitochondrial respiration for unregulated proliferation and survival, often in concert with downregulated glycolysis [3, 4, 5]. Therefore, there has been a recent paradigm shift towards understanding that cancer metabolic dependencies are not only variable across cancer subsets, but that they can also be measured to reveal cancer cell liabilities [6, 7]. Once identified and validated, these metabolic-pathway liabilities can be exploited for therapeutic targeting [8]. Promising druggable targets for cancer metabolic therapies include oncogenes that rewire energy metabolism, intermediates in oncogene pathways, and the genes, proteins, and pathways associated with substrate and nutrient transport and utilization/oxidation.

A paradigm shift: Heterogeneity in metabolic phenotype driving oncogenesis

Cancer cells undergo various metabolic changes as they acquire altered traits to adapt, survive, and metastasize in environments with varying substrate availability and oxygen concentration [9]. As a result, they display profound genetic, bioenergetic, and functional differences from their nontransformed parental cells. In the last decade, bioenergetic studies have highlighted the variability among cancer types, and even within cancer types, in the mechanisms and the substrates preferentially used for deriving this vital energy. While glycolysis (or Warburg metabolism) has long been considered the major metabolic process for energy production and anabolic growth in cancer cells, it is now clear that mitochondria play a key role in oncogenesis [10]. In addition to central metabolic and bioenergetic function, mitochondria also provide building blocks for tumor anabolism, as well as controlling redox and calcium homeostasis [6]. Although a main tenet of cancer is dysregulation of normal cell metabolism that contributes to abnormal cell growth, cancer is not one disease. Thus, understanding how the metabolic pathways of a given type of cancer are rewired is critical to identify and develop opportunities for therapeutic intervention.

Defining metabolic/bioenergetic phenotype

An initial step in understanding any type of cancer is to characterize the metabolic and bioenergetic signature of the cell. The Oxygen Consumption Rate (OCR)/Extracellular Acidification Rate (ECAR) ratio and Agilent Seahorse XF Cell Energy Phenotype Test (described in detail in Rogers et al, 2019 [11]) provide a high-level assessment of bioenergetic poise. Lanning et al. used this assay to show that triple-negative breast cancer (TNBC)-derived cell lines displayed significant heterogeneity with respect to basal metabolic phenotype (Figure 1) [12]. In a similar study, Guha et al. profiled the basal metabolic signatures of several breast cancer cell lines (Figure 2). Here, the authors showed that aggressive TNBC cells have a unique metabolic phenotype associated with mitochondrial genetic and functional defects [13]. These oncogenic defects impaired mitochondrial respiration and induced a metabolic switch to glycolysis, which is associated with tumorigenicity. These results suggest that metabolic phenotype could be used to identify TNBC patients at risk of metastasis and that altered metabolism can be targeted to improve chemotherapeutic response.

Based on OCR and ECAR XF measurements, a more functional and quantitative measure of the bioenergetic phenotype is to quantify the rate and source (mitochondrial versus glycolytic) of ATP production. The Agilent Seahorse XF Real-Time ATP rate assay measures the total ATP production rate in cells and distinguishes between ATP produced from mitochondrial oxidative phosphorylation (OXPHOS) and glycolysis (Figure 3 and [14]). When applied to a panel of cancer cell lines, this assay shows that cancer cell lines utilize mitochondrial OXPHOS and glycolytic activities differently to meet their energy demands (Figure 4 and [15]). These findings underscore the fact that different cancer cells adopt unique metabolic signatures that are critical to setting the strategy for targeting a given disease subtype and determining whether a given cell line is a good model for the disease of interest.

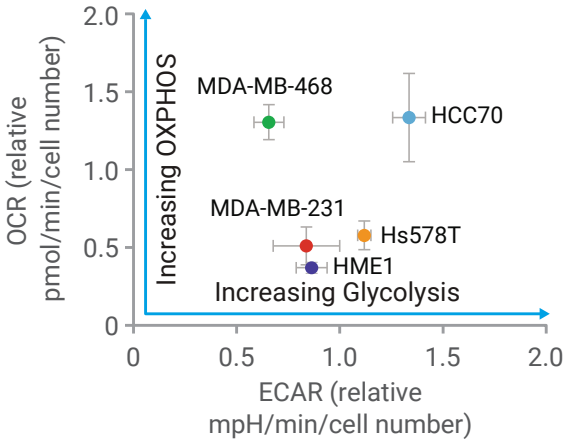

Figure 1. Agilent Seahorse XF energy map of breast cancer cell lines reveals different metabolic phenotypes. Normalized ECAR and OCR data were plotted to reveal overall relative basal metabolic profiles for each cell model [12].

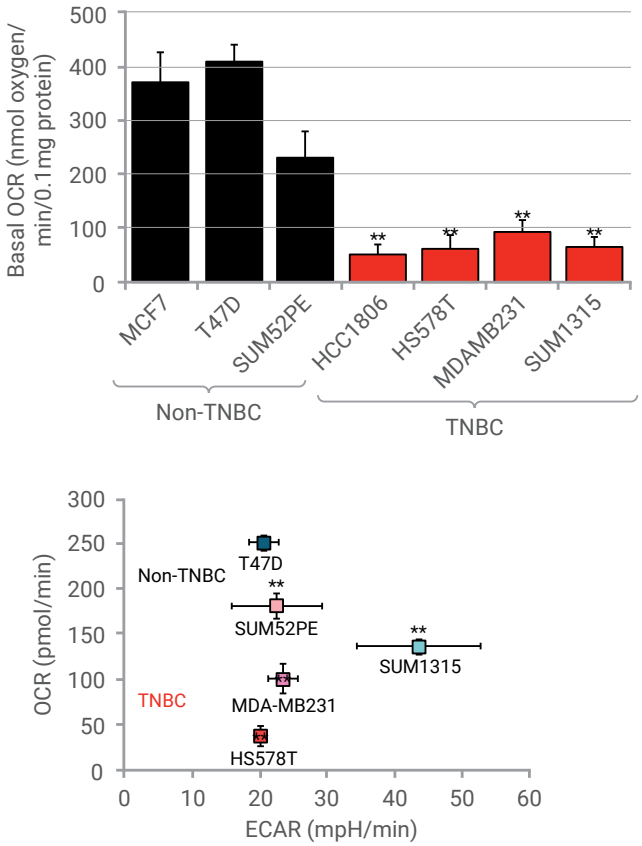

Figure 2. TNBC and non-TNBC cell lines display distinct metabolic phenotypes. Top: Basal cellular oxygen consumption rate (OCR) of TNBC- and non-TBNC cell lines. Bottom: XF energy map of TNBC and non-TNBC cell lines [13].

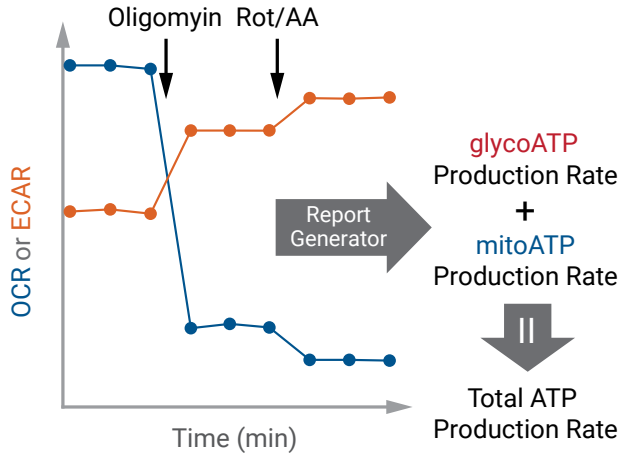

Figure 3. Representative scheme of the Agilent Seahorse XF Real-Time ATP rate assay. This assay provides quantitative measurements of ATP production rate from both mitochondrial and glycolytic pathways. Both OCR and ECAR of live cells are simultaneously measured using the Agilent Seahorse XF Analyzer. Using standardized control compound injections and data analysis, cellular ATP production rates are reported [14].

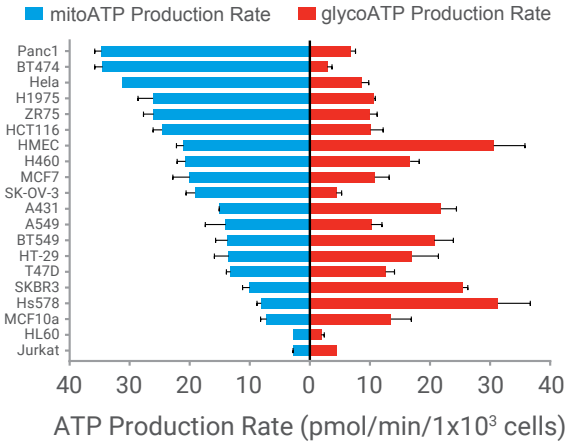

Figure 4. Cancer cells have developed different strategies for cellular energy production, with significant implications for therapeutic strategy. Measuring ATP production rates across a panel of 20 cancer cell lines reveals a wide range of energy phenotypes, from predominantly oxidative (top) to predominantly glycolytic (bottom) [15].

Modulating cancer cell energy metabolism

Measuring changes in cancer cell metabolism in response to potential therapeutic compounds is paramount for understanding drug mechanism of action, efficacy, toxicity, and off-target effects. When investigating effects of γ-Tocotrienol (γ-T3) on human gastric adenocarcinoma cells, Wang et al. observed a significant shift away from mitochondrial and towards glycolytic ATP production. Further, γ-T3 reduced the total ATP production rate in these cells (Figure 5) [16].

Once a change in bioenergetic phenotype is detected, further investigation often involves uncovering the mechanism responsible for the shift in metabolic poise. Thus, to gain insight into observations presented in Figure 5, further assays were performed to directly assess mitochondrial and glycolytic function in the presence of γ-T3. Using the Agilent Seahorse XF Cell Mito Stress Test, Wang et al. demonstrated dose-dependent decreases in basal and maximal respiration for both SGC-7901 and MGC-803 cells, both indicative of mitochondrial dysfunction (Figure 6). Using the Agilent Seahorse XF Glycolytic Rate Assay to provide a quantifiable measurement of glycolysis (Figure 7 [17]), the authors also showed a dose-dependent increase in basal glycolysis in both cell types (Figure 8). This data supports the observation of cellular ATP production switching predominantly to glycolysis, but at a lower total production rate. These results, with orthogonal data, suggest that mitochondrial function may be an effective target for anticancer drug development efforts [16].

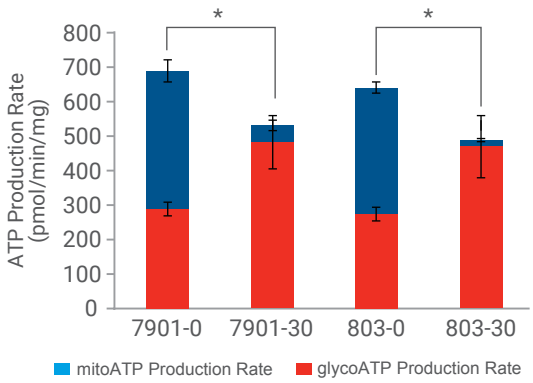

Figure 5. Quantification of ATP production rate reveals a drug-induced glycolytic switch in two adenocarcinoma cell lines. Gastric cancer cells were treated with 0 or 30 µM γ-T3 for 4 h, and the ATP production rate was measured using the Agilent Seahorse XF Real Time ATP rate assay. Adapted from [16].

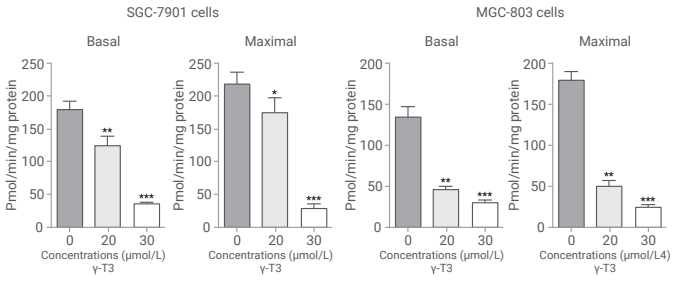

Figure 6. Dose-dependency of the effect of γ-T3 on mitochondrial function is measured by the Agilent Seahorse XF Cell Mito Stress Test. SGC-7901 and MGC-803 cells were treated with indicated concentrations of γ-T3 for 4 h, then basal and maximal OCR were measured using the XF Cell Mito Stress Test [16].

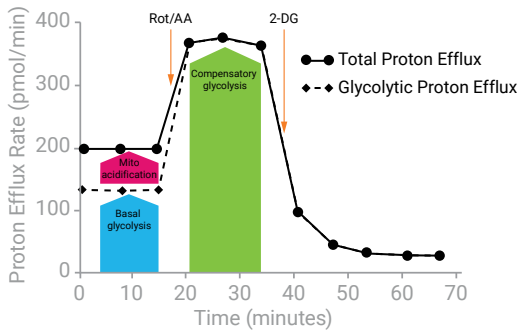

Figure 7. Representative scheme for the Agilent Seahorse XF Glycolytic Rate Assay. This assay provides a quantitative measure of glycolysis using both OCR and ECAR (simultaneously measured by the Agilent Seahorse XF Analyzer) to measure and subtract mitochondrial acidification. The resulting glycolytic Proton Efflux Rate (glycoPER) under both basal and compensatory conditions are reported [17].

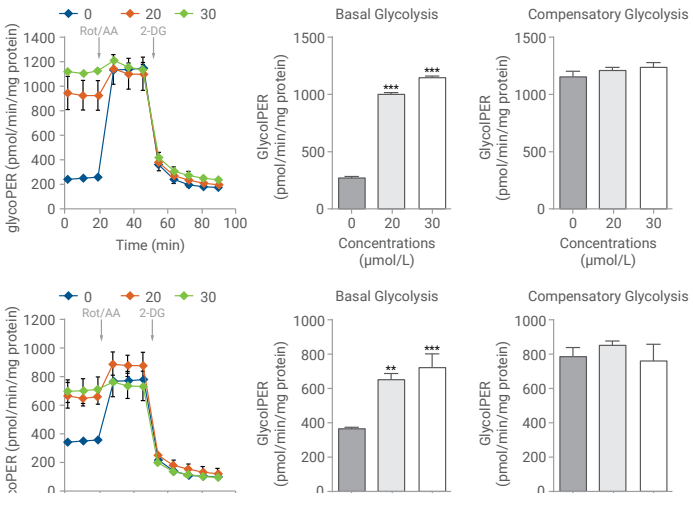

Figure 8. Dose-dependency of the effect of γ-T3 on the glycolytic rate is measured by the Agilent Seahorse XF Glycolytic Rate Assay. SGC-7901 (top) and MGC-803 (bottom) cells were treated with indicated concentrations of γ-T3 for 4 h and the extracellular acidification rates were measured using the XF Glycolytic Rate Assay. Bar graphs show quantitative data of basal and compensatory glycolysis rates [16].

Mitochondrial and glycolytic function as therapeutic targets: the genotype-metabolic phenotype connection.

Beyond initial classification of cancer cell energetic phenotypes, ATP production rates and the proportion of ATP sourced from OXPHOS versus glycolysis can be further used to investigate the functional effects of cancer-associated mutations. For example, evaluation of KRAS-and EGFR-mutated NSCLC cells showed distinct metabolic phenotypes concerning the source of ATP production (Figure 9 [18]). The cell lines bearing EGFR mutations were, in general, more oxidative than those bearing KRAS mutations, providing insight into metabolic vulnerabilities of cancer subsets, and EGFR as a potential therapeutic target.

A study by Deribe et al. provides another example of how measurements with the XF Cell Mito Stress Test revealed mutations of well-known cancer-causing genes, which could serve as a target for metabolic modulation [19]. In this study, the authors showed that mutations in the SWI/SNF chromatin remodeling complex induce a targetable dependence on OXPHOS in lung cancer cell lines. Cells deficient in the most frequently inactivated complex subunit, SMARCA4 (KPS cells, Figure 10A, B), had increased mitochondrial respiration. When the SMARCA4 deficient H1299 lung cell line was reconstituted with SMARC4A (H1299 SMARCA4), both basal respiration and spare respiratory capacity decreased (Figure 10 C, D), further showing that these mutant lung cancer cells are sensitive to inhibition of OXPHOS [19].

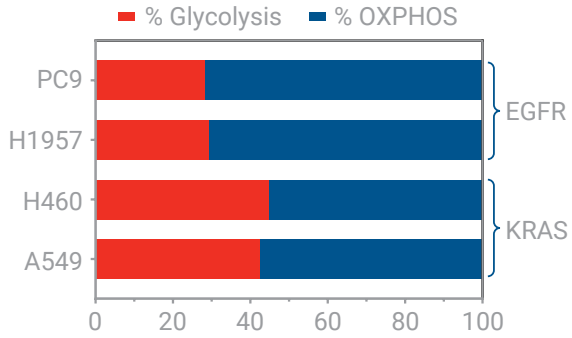

Figure 9. KRAS-and EGFR-mutated NSCLC cells exhibit different ATP production patterns. Metabolic profiles of four cellular models of NSCLC in standard assay medium (XF RPMI containing 10 mM glucose, 1 mM pyruvate, 2 mM glutamine, pH 7.4) These two KRAS mutant cell lines are slightly more glycolytic than the EGFR mutant cell lines [18].

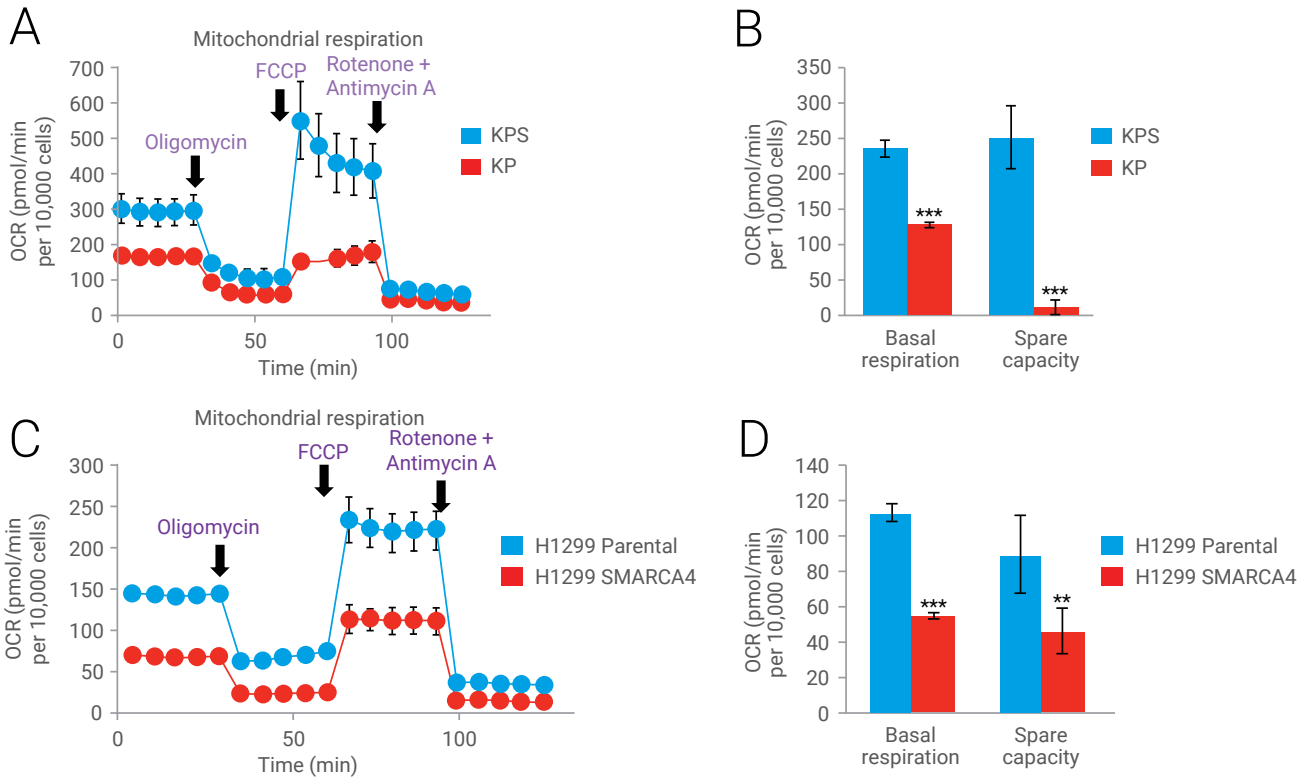

Figure 10. Measuring the functional effect of mitochondrial gene mutations using basal respiration and spare respiratory capacity. A) trace of OCR values from a mitochondrial stress test showing Kras-p53 (KP) -derived cell line (red) and Kras-p53-SMARCA4 -derived cell line (KPS , i.e. SMARCA4 deficient, blue). B) basal respiration and spare respiratory capacity. C) kinetic trace of the mitochondrial stress test showing H1299 parental (i.e. SMARCA4 deficient, blue) and H1299 cell line reconstituted with SMARCA4 (red). D) basal respiration and spare respiratory capacity [19].

The previous examples have discussed how XF measurements and assays revealed metabolic vulnerabilities with upregulated OXPHOS. Similarly, metabolic vulnerabilities associated with upregulated glycolysis can be identified using In another example of oncogenes driving changes in metabolic phenotype, Barnoud et al. recently demonstrated that tumor cells expressing a p53 allele with a serine at amino acid 47 (S47 tumor cells) exhibited upregulated glycolysis with reduced dependency on OXPHOS compared to cell with wild type p53 [20]. This was accomplished by performing both XF Glycolytic Rate and XF Mito Stress Test assays to investigate glycolytic and mitochondrial function, respectively. Measurement of mitochondrial function revealed decreased basal and maximal (stressed) mitochondrial respiration of S47 cells compared to wild type cells (Figure 11A), suggesting a decreased ability to perform OXPHOS. In contrast, measurement of basal and compensatory glycolysis (Figure 11 B–D) showed that the S47 tumor cells were upregulating glycolysis under both basal and stressed conditions. This indicates that metabolic rewiring resulted in a greater dependency on glycolysis compared to the wild type. Taken together, the results suggest that in these tumor cells, the glycolytic pathway is a potential metabolic target for S47 variant cancers. This is further supported by experiments showing increased sensitivity of S47 cells to the glycolytic inhibitor, 2-deoxyglucose [20].

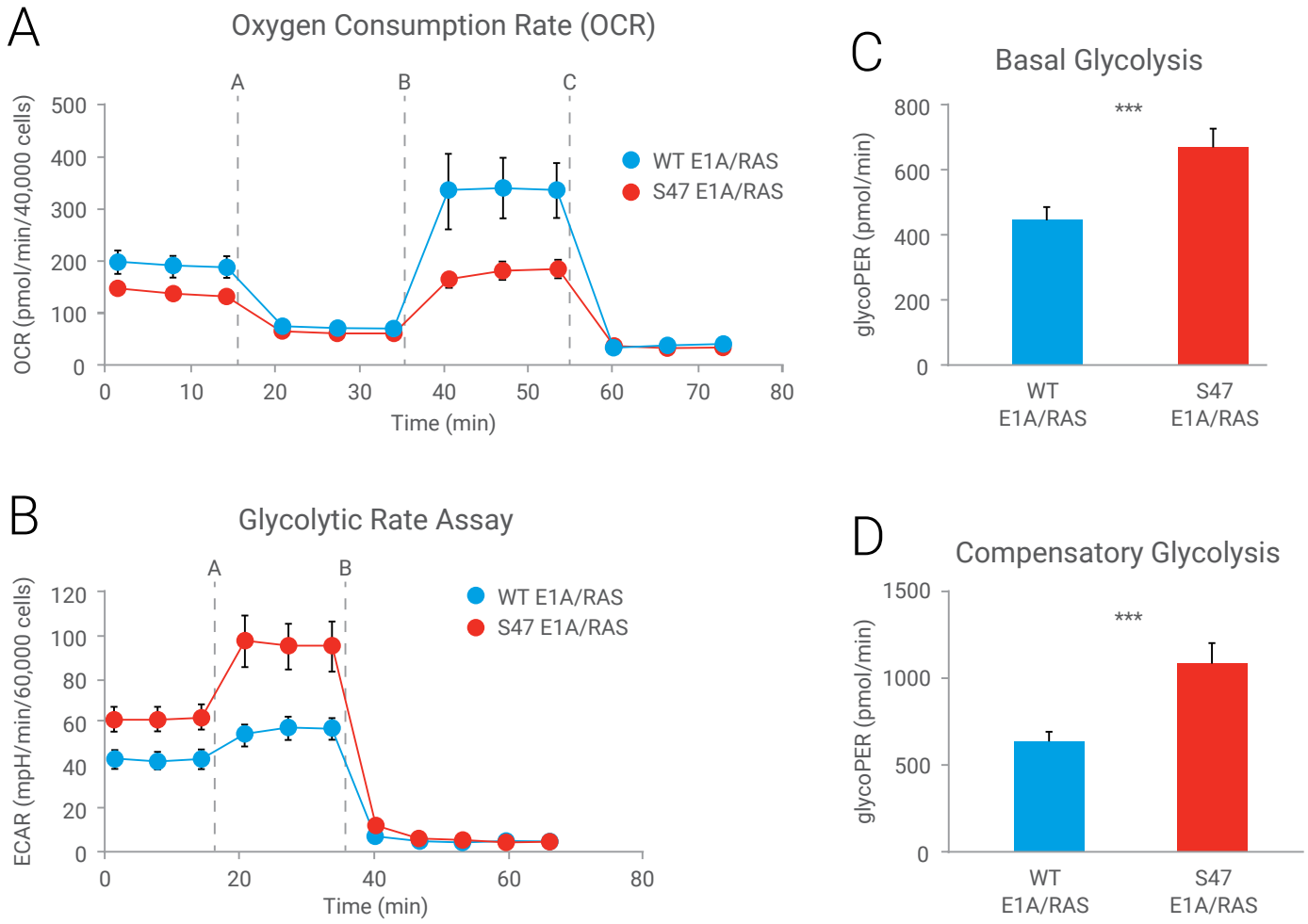

Figure 11. Metabolic changes in tumor cells induced by a p53 allele with a serine at amino acid 47 (S47 tumor cells). A) The Agilent Seahorse XF Cell Mito Stress Test reveals a decrease in basal mitochondrial respiration and spare respiratory capacity in S47 tumor cells compared to wild type cells. B, C, D) The Agilent Seahorse XF Glycolytic Rate Assay reveals a statistically significant difference in basal and compensatory glycolysis for S47 tumor cells compared to WT cells (Figure adapted from [20]).

Summary

- To first understand changes in the bioenergetic poise between oxidative phosphorylation and glycolysis in cancer cells, it is recommended to measure the OCR/ECAR ratio. This metric is an easy-to-use indicator of changes in cell phenotype or metabolic activity. Assays that can be used to determine this ratio include the XF Cell Energy Phenotype Test, the XF Cell Mito Stress Test, and the XF Glycolytic Rate Assay.
- A more functional and quantitative measure of cancer cell metabolic phenotype is to quantify the rate and source (mitochondrial versus glycolytic) of ATP production. The XF Real-Time ATP rate assay measures the total ATP production rate in cells and distinguishes between ATP produced from mitochondrial OXPHOS versus glycolysis. This assay therefore provides a detailed, functional overview of cancer cell phenotype and metabolism.
- Used in combination the quantitative and functional parameters provided by the XF Real-Time ATP rate assay (total, glyco-, and mitoATP production rates), the XF Cell Mito Stress Test (basal and maximal OCR), and the XF Glycolytic Rate Assay (basal and compensatory glycolysis) can reveal the effects of anticancer agents in terms of metabolic liabilities, efficacy, toxicity, target identification, mechanism of action, etc.
- There is increasing evidence for a correlative pattern among oncogenes (i.e. the expressed protein) and effects on cancer cell metabolism regarding changes in mitochondrial and glycolytic function and the ATP production rate/source. These metabolic changes or shifts can be potential targets for anticancer therapeutics.

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Printed in the USA, May 24, 2019
5994-1017EN

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