Cancer Cells Evoke Flexibility By Maintaining a Bioenergetic Balance Between Glycolysis and Mitochondrial Respiration Linked to Metastatic Potential

Application Note

Introduction

Alterations in cellular metabolism are a crucial hallmark of cancer. With regard to the progression from primary tumor through local invasion to distal metastatic implantation and finally late stage tumor growth, cancer cell metabolism changes from mitochondrial respiration to metabolic symbiosis to aerobic glycolysis. This progression was studied and discussed in a series of papers listed below. The cancer cells used in this analysis include several different cancer cell lines: 1) SH-SY5Y, a neuroblastoma cell line, derived from a primary tumor, 2) primary melanoma cells and cell lines, and 3) PC3M, a prostate cell line, best described as a late stage tumor.

Cellular metabolism has been described by three different phenomena:

- The Pasteur Effect, which describes the situation in which increasing oxygen availability reduces glucose consumption[4]
- The Crabtree Effect, which describes the phenomenon in which increasing glucose availability reduces oxygen consumption[5]
- The Warburg Effect, which applies in tumor cells, in which glucose is consumed regardless of the amount of oxygen consumed[6]

Of these three scenarios, tumor cells are highly glycolytic such that even in the presence of oxygen they produce free protons at a higher rate than normal cells, as described above (lactate production from glucose, the Warburg effect). As a consequence, the microenvironment of solid tumors is acidic, which in turn significantly affects tumor growth and local invasion. Specifically, low extracellular pH leads to increased release of proteolytic enzymes that result in degradation of the extracellular matrix (ECM). These observations have led to the acid-mediated invasion hypothesis, which proposes that H^+ flows along concentration gradients from the tumor into peritumoral normal tissue causing normal cell death and ECM degradation. Cancer cells, which are acid-adapted, are then able to invade into the damaged adjacent normal tissue and metastasize.

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Results and Discussion

It is widely accepted that metabolic changes are one of the hallmarks of cancer, and aerobic glycolysis is a major source of energy in malignant cells, but mitochondrial involvement via oxidative phosphorylation contributes significantly as measured by oxygen consumption rate (OCR) in melanoma compared other cancer types, and metabolic symbiosis (lactate produced by glycolysis in the central hypoxic areas fuels ATP production via OXPHOS in the mitochondria in the normoxic regions).

The metabolic shift from mitochondrial respiration to glycolysis as the primary source of cellular energy is revealed quite specifically as cells progress from HEMs (human epidermal melanocytes) to malignant melanomas. Key enzymes associated with high OXPHOS are substantially elevated in primary and metastatic melanomas compared to nevic melanocytes. LDH1 and LDH2 isoforms play a major role in the production of pyruvate from lactate, supporting the bioenergetic pathway of OXPHOS. Compared to HEMs, melanoma cells are more metabolically active and, in addition to glycolysis, derive a significant proportion of energy from OXPHOS, which, in the progression to advanced melanoma, correlates with increased metabolic flexibility (Figure 1B). Patients with advanced metastatic melanoma and normal serum LDH use OXPHOS and glycolysis equally, while patients with advanced metastatic melanoma and high serum LDH have a poor prognosis. Their LDH profile indicates that they have elevated levels of LDH3 and LDH4, suggesting that glycolysis is the primary metabolic pathway used by tumor cells.

Using prostate cancer cells, PC3M, Ibrahim-Hashim; *et al.*[3], demonstrated that the shift to glycolysis yielded a high extracellular acidification rate (ECAR) level and provided local acidosis (Figure 2A). High acid production of the PC3M cells supports rapid destruction of the normal stromal cells surrounding the tumor site, invasion, and metastasis. This tumorigenesis process could be disrupted by treatment with 200 mM free lysine, which neutralizes the acidic conditions,

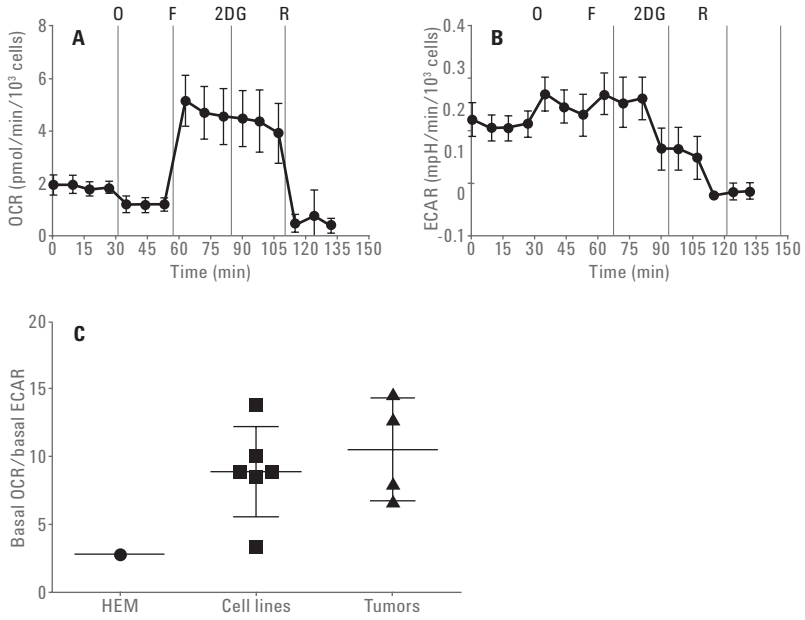

Figure 1. From Ho; *et al.*[2]: Bioenergetics analysis in single-cell melanoma suspensions, melanoma cell lines and HEMs (A) single-cell suspensions from a fresh tumor respond to pharmacologic inhibitors of metabolism, as determined by a Agilent Seahorse XF24 Extracellular Flux Analyzer. Depicted are OCR (A) and ECAR (B) rates of single-cell suspensions, prepared from one of four metastatic melanomas. Similar results were obtained with the other three tumors. After baseline OCR and ECAR determination, the melanoma cells were treated with oligomycin (O), FCCP (F), rotenone (R), or 2-DG. (B) Ratio of basal OCR over basal ECAR in HEMs, melanoma cell lines, and single-cell melanoma suspensions. Data are depicted as a ratio of basal OCR over basal ECAR ratio of short-term cultures of HEMs, six different metastatic human melanoma cell lines (WM983-A, WM983-B, WM1158, WM852, Lu1205 and C32) and single-cell suspensions obtained from four subcutaneous metastatic melanomas.

reducing tumor growth and diminishing metastasis compared to treatment with tap water. Further, animals treated with the free lysine lived longer than the animals that received just tap water injections.

With respect to the SH-SY5Y neuroblastoma cell line, under conditions of fixed energy demand ECAR (Figures 3A and 3B) and OCRs (Figures 3C and 3D) values showed a reciprocal relationship. In addition to observing an expected Crabtree effect in which increasing glucose availability raised the ECAR and reduced the OCR, a novel reciprocal relationship was documented in which reducing the ECAR via glucose deprivation or glycolysis inhibition increased the OCR. It has been shown that reduced ECAR conditions affect proteins that associate with energy sensing and energy response pathways. ERK phosphorylation, EIRT1, and HIF1a decreased while AKT, p38, and AMPK phosphorylation increased (data not shown). That is, restricting glycolytic flux increases mitochondrial respiration.

Glycolysis and glycolytic capacity can only be measured in whole cells, a measurement enabled by the use of the Agilent Seahorse XF Extracellular Flux Analyzer (Billerica, MA). To enable these measurements, the SH-SY5Y neuroblastoma cells were cultured for two hours in the absence of glucose. Complete cellular glycolytic capacity was determined by treating cultures with addition of 2-deoxyglucose (100 mM), an inhibitor of the first step of glycolysis. The ECAR measured was a result of glycolytic metabolism. The addition of oligomycin (1 µM), an inhibitor of ATP synthase, reduces mitochondrial respiration and maximizes glycolytic ATP production (Figure 2).

Not much is known regarding the importance of crucial metabolic pathways in melanoma development and progression. What we have learned from the paper by Ho; *et al.*[2] is that patients who have high serum LDH levels have elevated levels of LDH isoenzymes, which drives pyruvate conversion to lactate. Secondly, enzymes associated with glycolysis as well as OXPHOS are expressed at higher levels in primary and metastatic melanomas, which suggests that the progression to advanced melanoma and increased metabolic flexibility are correlated. Third, in melanoma cells directly from patients, OXPHOS plays an important role in the generation of ATP. Finally, the data presented here document that MCT1 and MCT4 play an important role in the transport of carbon sources, including lactate, in response to environmental cues such as pH and oxygen tension. Further, the switch to glycolysis in advanced melanoma cells is due to their hypoxic state. To meet the high metabolic demands associated with melanoma development, proliferation, migration and invasion, both OXPHOS and glycolysis are upregulated in these cells.

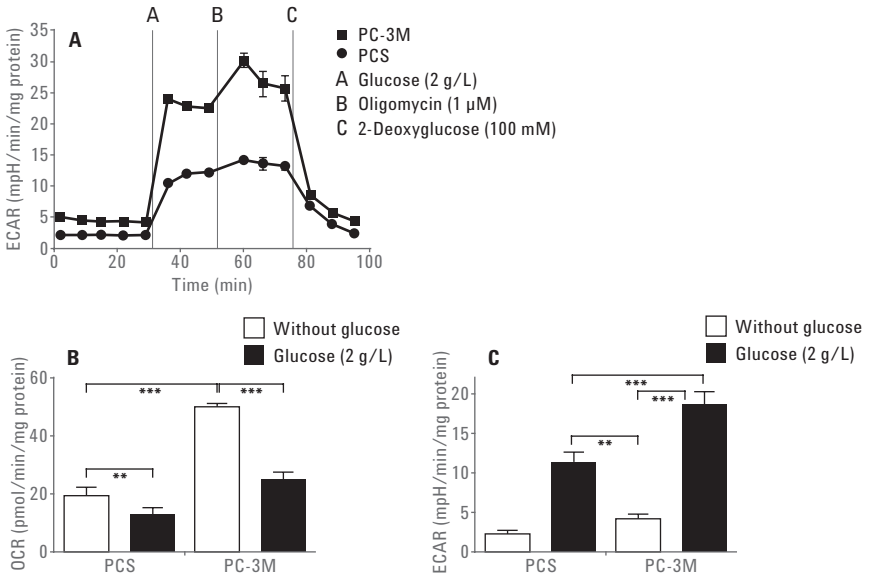

Figure 2. From Ibrahim-Hashim; *et al.*[3]: A) The glycolytic activity and maximum glycolytic capacity was determined for PC3M ad PCS cells in real-time using the Agilent Seahorse Extracellular Flux Analyzer. A series of extracellular acidification rates (ECAR) were calculated for cells glucose starved for two hours and subsequently treated with 2 g/L D-glucose (A), 1 µM oligomycin (B) and 100 mM 2-deoxyglucose (2-DG) (C). ECAR following the addition of glucose defines glycolysis and ECAR following oligomycin represents maximum glycolytic capacity. ECAR prior to the addition of glucose and following treatment with 2-DG represents acidification associated with non-glycolytic activity. The data represent the mean + standard deviation. (B-C) Basal oxygen consumption rates (OCRs) and extracellular acidification rates (ECARs) were determined for PC3M and PCS cells either in the presence or absence of 2 g/L D-glucose. The data represent the mean + standard deviation. A two-tailed Student's t-test was used to calculate statistical significance: **p <0.001 and ***p <0.0001.

Although the glycolytic phenotype is thought to be a near-universal phenomenon in cancer cells, it has not been specifically been described in PC3M. Therefore, Ibrahim-Hashim; *et al.*[3] first investigated the metabolic state of PC3M cells in comparison to a normal prostate cell line (PCS) *in vitro*. Real-time basal metabolic measurements of oxygen consumption and proton production were significantly higher in PC3M cells, suggesting that prostate cancer cells are more metabolically active than normal prostate cells. PC3M cells were found to have a greater glycolytic capacity in the presence of glucose and following inhibition of mitochondrial ATP production, implying that PC3M cells have the ability to depend on glycolysis much more so than PCS cells to meet energetic demands. Further, the cells respond to the lysine treatment without metastasis, and with prolonged survival.

In the Swerdlow paper, the authors demonstrate and characterize the phenomenon through which reduced glycolytic flux increases mitochondrial oxygen consumption based on the inverse relationship that occurs in these relatively immature neuroblastoma cells, SH-SY5Y, between glycolysis and mitochondrial respiration. The authors go on to predict that shifting the cell bioenergetic flux from glycolysis to respiration would impact pathways and proteins that regulate cell energy levels and redox states. An analysis of several relevant proteins, obtained under glucose starvation conditions, shows this does in fact occur for AKT, p38, and AMPK; all showed an increase in phosphorylation (data not shown), while a decrease in phosphorylation was seen for ERK, EIRT1, and HIF-1[*alpha*].

Alterations in cellular metabolism are a crucial hallmark of cancer. With regard to the progression from primary tumor through local invasion to distal metastatic implantation and finally late stage tumor growth, cancer cell metabolism changes from mitochondrial respiration to metabolic symbiosis to aerobic glycolysis.

References

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Additional Reading

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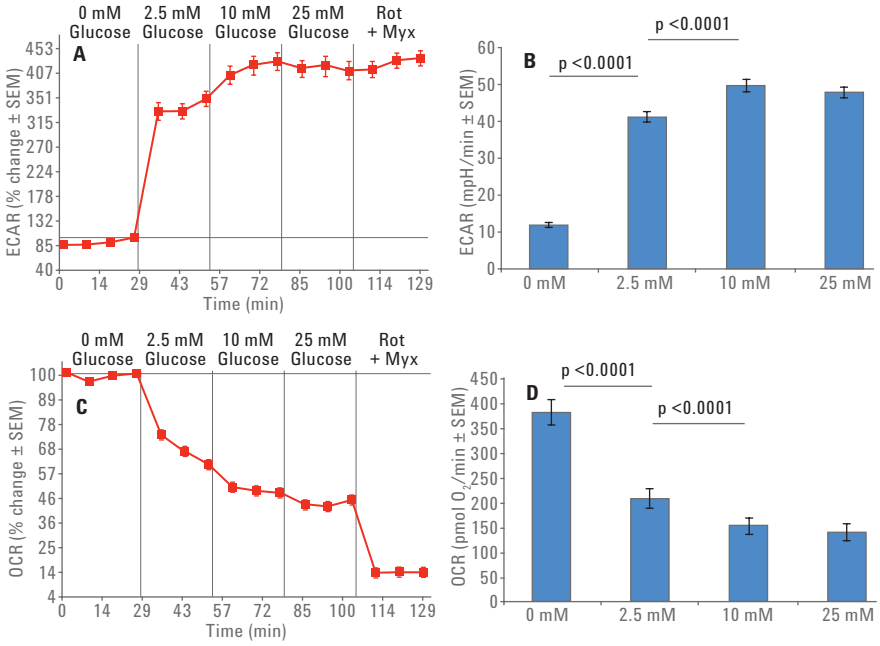

Figure 3. From Swerdlow; *et al.*[1]: Adding glucose to glucose-deprived SH-SY5Y cells induces a Crabtree effect. The change in the ECAR is shown as a percent change from baseline (A), and the absolute ECAR values under the different glucose conditions are shown in (B). The change in the OCR is shown as a percent change from baseline (C), and the absolute OCR values under the different glucose conditions are shown in (D). Rot = rotenone, Myx = myxothiazol.

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