

Energy metabolism and radiosensitivity of two HNSCC tumor cell lines

Christian G. Fabian, Wolfgang Mueller-Klieser, Ulrike G. A. Sattler

Institute of Physiology and Pathophysiology, University Medical Center of the Johannes Gutenberg University Mainz, Germany

Introduction:

Unlike normal tissue, most tumors show an increased glycolytic flux and an enhanced accumulation of lactate even in the presence of oxygen. This phenomenon, termed "aerobic glycolysis" or "Warburg effect", is mainly caused by an upregulation and/or transactivation of glycolysis-related enzymes and transporters (Moreno-Sanchez et al., FEBS J, 2007). Previous studies showed that lactate accumulation in tumors is associated with a high incidence of distant metastases, local recurrence and poor survival of patients (Brizel et al., 2001; Walenta et al., 2000). Furthermore, lactate concentrations were positively correlated with radioresistance in human head and neck squamous cell carcinoma (HNSCC) xenografts (Sattler et al., Radiother Oncol, 2010).

In the present experimental study, two HNSCC cell lines (UT-SCC-8 and SAS) were characterized regarding metabolic, energetic and radiobiological properties (Figure 1).

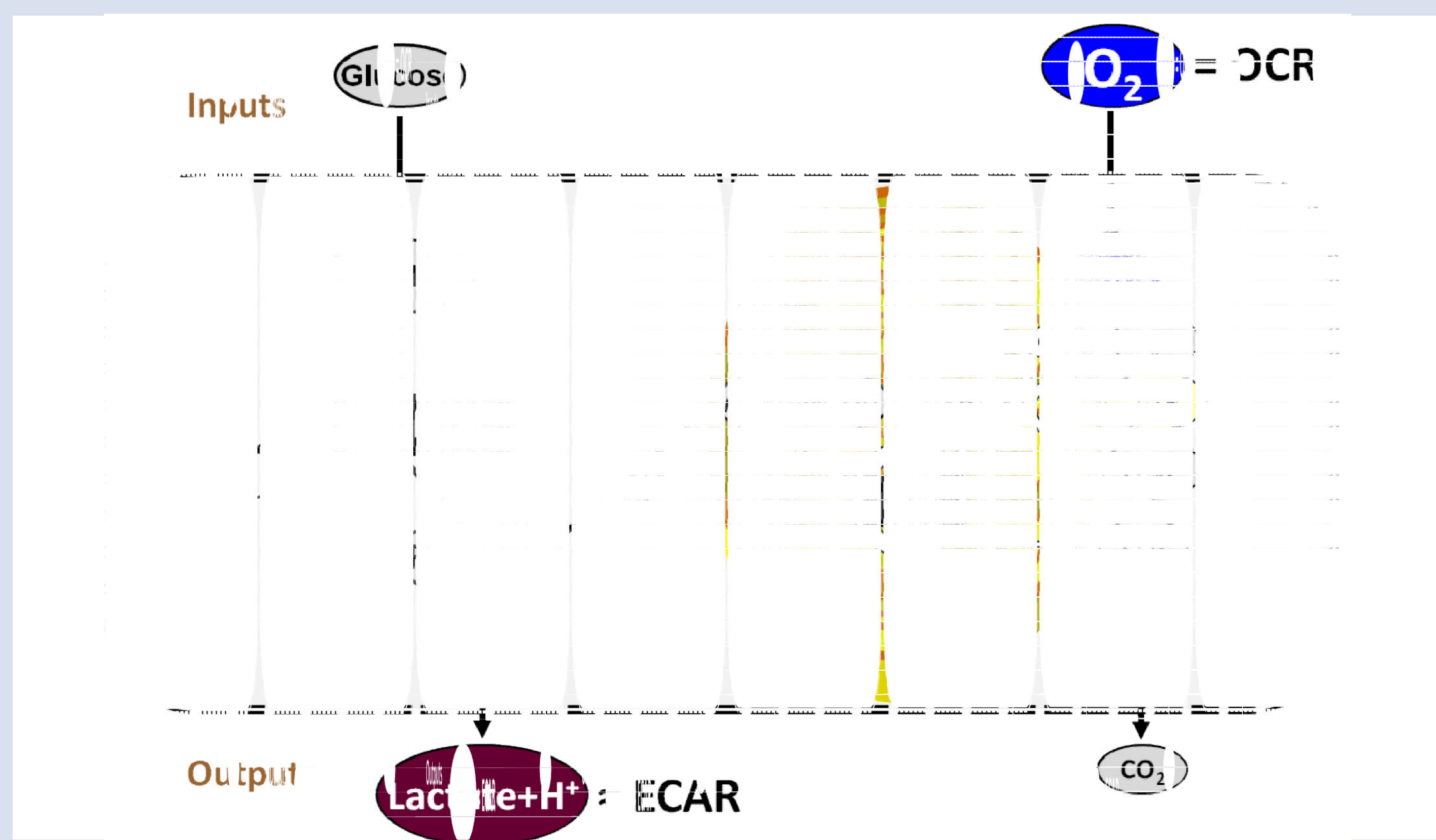


Figure 1: Diagram of the tumor metabolism including measurement parameters: Extracellular acidification rate (ECAR) and oxygen consumption rate (OCR)

Materials and Methods:

Lactate production was measured in medium supernatants with a commercial photometric test (R-biopharm, Darmstadt, Germany). Therefore, cells were seeded in six-well plates. After 3 days, the medium was changed and medium supernatants were collected for lactate measurement at indicated times (0, 2, 4, 6, 12, 18, 24h). Lactate concentrations were normalized to cell volumes which were assessed by an automatic cell counter (CASY Technology, Reutlingen, Germany).

Proton release and oxygen consumption were quantified with the Seahorse extracellular flux analyzer XF24 (Seahorse Bioscience, Billerica, MA). It determines non-destructively and non-invasively the extracellular acidification rate (ECAR) and oxygen consumption rate (OCR) in the supernatant of living cells in real time. The measurements are performed using fluorescent biosensors, whose fluorescent signal is quenched by the presence of the specific analyte. UT-SCC-8 and SAS were seeded at 50,000 cells per well in 450 μ l complete DMEM growth medium and incubated for 48h (UT-SCC-8) and 24h (SAS) at 37°C/5% CO₂, respectively. The XF assay was initiated by replacing the growth medium with an unbuffered assay medium (D5030, Sigma-Aldrich, St. Louis, MO) supplemented with 1 g/l glucose. XF24 analyzer results were normalized to Janus Green staining to adjust for cell plating differences.

Colony forming assays were accomplished from cells irradiated with single doses of 0, 2, 4 and 8 Gy. After irradiation, cells were seeded in culture dishes and incubated at 37°C/5% CO₂ for 10 (UT-SCC-8) and 5 (SAS) days, respectively. Subsequently, colonies were stained with methylene blue (Merck KGaA, Darmstadt, Germany) and were quantified by computerized image analysis (ImageJ).

Results:

The HNSCC tumor cell lines investigated showed an explicit difference in lactate production, most obviously within the first 6h (Figure 2). After 24h UT-SCC-8 produced 4.37 ± 0.57 mol lactate/liter cell volume (mol/l). This was lower than that of SAS cells (5.51 ± 0.45 mol/l). For the determination of the lactate production rate, expressed as mol lactate per liter cell volume per hour (mol/l/h), the first 6 hours of the experiments were taken into account. Consequentially UT-SCC-8 cells revealed a significantly lower ($p < 0.05$) production rate of 0.24 ± 0.05 mol/l/h compared to SAS cells with 0.34 ± 0.04 mol/l/h (Figure 2 right panel).

References:

- Moreno-Sanchez, R, Rodriguez-Enriquez, S, Marin-Hernandez, A, Saavedra, E (2007). Energy metabolism in tumor cells. FEBS J 274; 1393–1418.
Brizel, DM, Schroeder, T, Scher, RL, Walenta, S, Clough, RW, Dewhirst, MW und Mueller-Klieser, W (2001). Elevated tumor lactate concentrations predict for an increased risk of metastases in head-and-neck cancer. Int J Radiat Oncol Biol Phys 51; 2:349-353.
Walenta, S, Wetterling, M, Lehrke, M, Schwicker, G, Sundfor, K, Rofstad, EK und Mueller-Klieser, W (2000). High lactate levels predict likelihood of metastases, tumor recurrence, and restricted patient survival in human cervical cancers. Cancer Res 60; 4:916-921.
Sattler, UGA, Meyer, SS, Quenett, V, Hoerner, C, Knoerzer, H, Fabian, C, Yaromina, A, Zips, D, Walenta, S, Baumann, M, Mueller-Klieser, W (2010). Glycolytic metabolism and tumour response to fractionated irradiation. Radiother Oncol 94; 102–109.

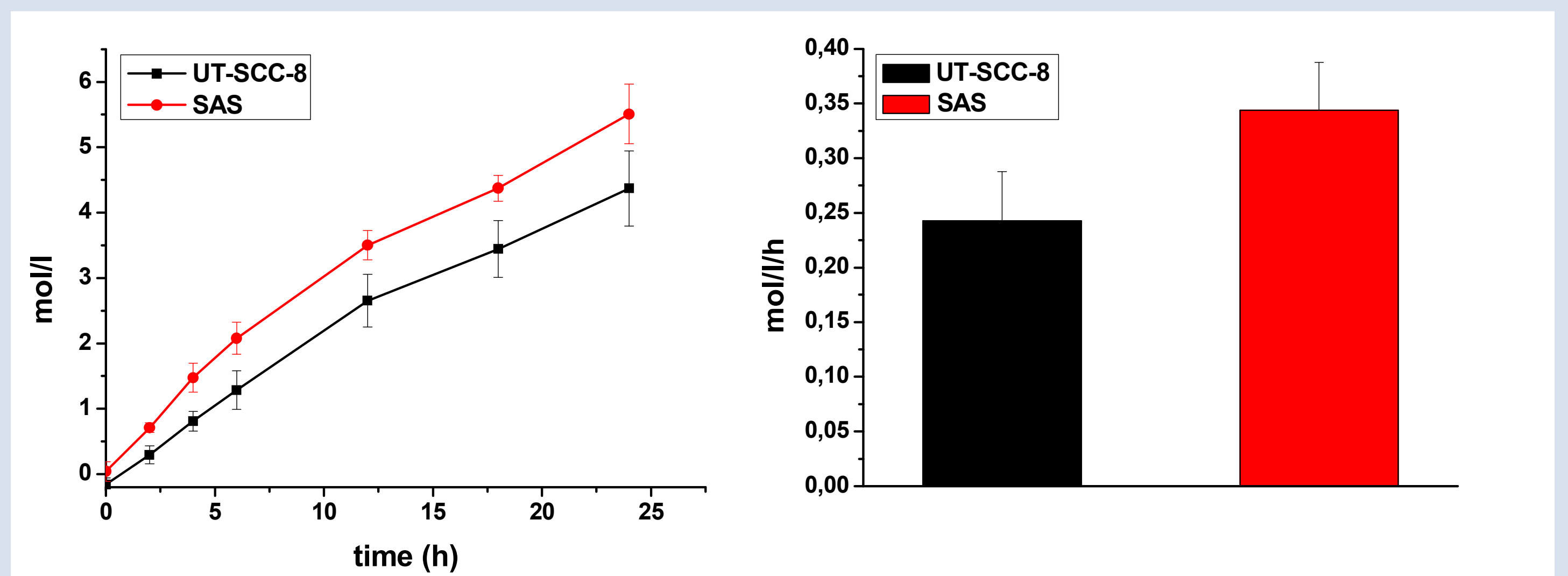


Figure 2: Kinetics of lactate production

Left: After 24 hours, UT-SCC-8 and SAS cells showed a lactate production of 4.37 ± 0.57 mol lactate/liter cell volume and 5.51 ± 0.45 , respectively.

Right: Lactate production rate; 0.24 ± 0.05 and 0.34 ± 0.04 mol lactate/liter cell volume/h for UT-SCC-8 and SAS, respectively.

Each sample was analyzed in triplicate and mean \pm SD of three independent experiments are shown.

Accordingly, extracellular flux analyses revealed a significantly lower ($p < 0.05$) extracellular acidification rate (ECAR) for UT-SCC-8 (27.7 ± 8.5 mpH/min) compared to SAS (54.9 ± 9.7 mpH/min). Oxygen consumption was significantly lower ($p < 0.05$) in UT-SCC-8 (81.8 ± 24.0 pmol/min) than in SAS (114.1 ± 18.8 pmol/min). Thus, SAS cells are characterized by an intensified glycolytic and oxidative metabolism compared to UT-SCC-8 cells (Figure 3).

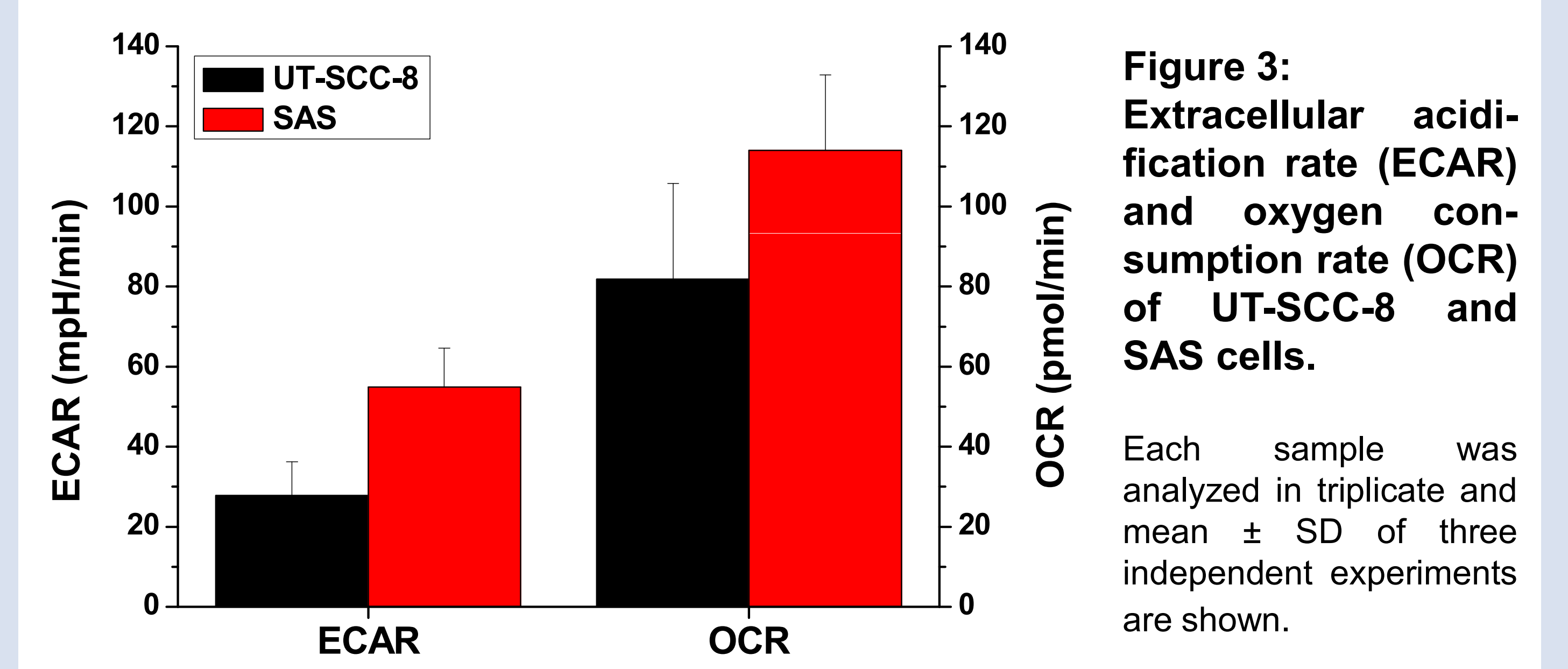


Figure 3: Extracellular acidification rate (ECAR) and oxygen consumption rate (OCR) of UT-SCC-8 and SAS cells.

Each sample was analyzed in triplicate and mean \pm SD of three independent experiments are shown.

SAS cells showed higher ECAR as well as higher OCR compared to UT-SCC-8.

For a 10% clonogenic cell survival of UT-SCC-8 and SAS cells doses of 5.8 and 7.7 Gy gamma irradiation respectively were obtained from dose response curves (Figure 4).

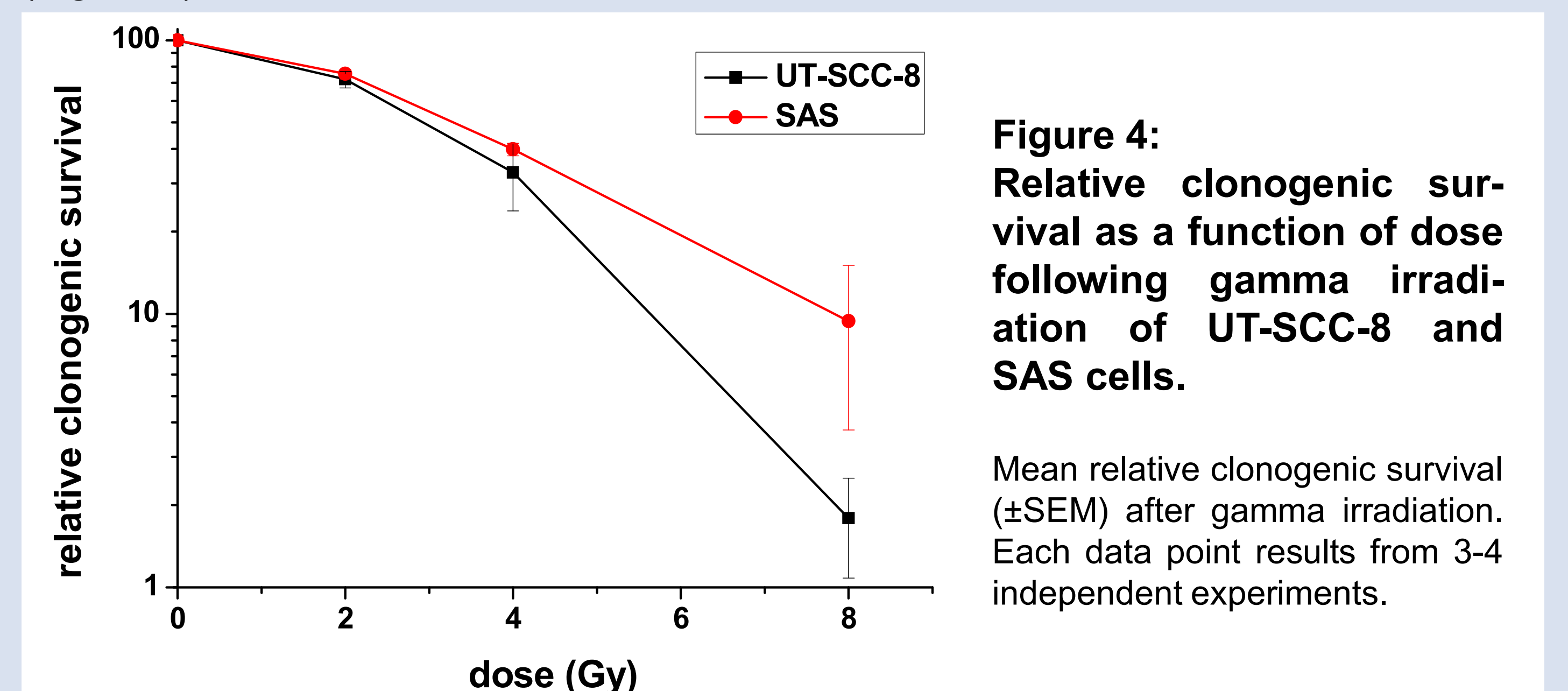


Figure 4: Relative clonogenic survival as a function of dose following gamma irradiation of UT-SCC-8 and SAS cells.

Mean relative clonogenic survival (\pm SEM) after gamma irradiation. Each data point results from 3-4 independent experiments.

SAS cells are more resistant to irradiation than UT-SCC-8.

Discussion:

The data presented support the hypothesis that enhanced lactate production may be associated with an intracellular accumulation of lactate and other glycolytic intermediary products. Due to the radical scavenger function of some of these products they may confer radioresistance to highly glycolytic cells. The results obtained encourage more research efforts to be directed towards targeting tumor glycolysis for therapeutic radiosensitization.

Summary:

- Lactate production of SAS cells is higher than that of UT-SCC-8 cells
- ECAR and OCR are higher for SAS cells than for UT-SCC-8
- Clonogenic cell survival is higher in SAS cells compared to UT-SCC-8
- Tumor cell metabolism may be associated with radioresistance, on the cellular level