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Manipulation of lactate metabolism and its impact on radiation resistance in two human ovarian cancer cell lines

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Introduction:

Malignant transformation is associated with an increase in glycolytic flux and an enhanced accumulation of lactate even under normoxic conditions. This phenomenon, termed "Warburg effect", is mainly caused by an upregulation of numerous glycolytic and glycolysis-related genes in the majority of human tumors[1]. Therefore, manipulation of the glycolytic pathway may alter tumor cell metabolism and thereby influence its radiobiological properties.

In the present experimental study, two human ovarian cancer cell lines IGROV-1 and OC316, were characterized according to their metabolic and radiobiological properties with and without addition of oxamate (OXA) and 2-Deoxy-D-glucose (2-DG). Oxamate is a structural analog of pyruvate and a competitive inhibitor of lactate dehydrogenase (LDH)[2]. Furthermore oxamate may have an antioxidative character due to its structural similarity with pyruvate[3]. 2-Deoxy-D-glucose is a competitive inhibitor of hexokinase and decreases glucose metabolism[4].

Materials and Methods:

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. To investigate the influence of 20-60 mM oxamate and 5-40 mM 2-Deoxy-D-glucose, inhibitors were injected at different time points directly to the cells. Results were normalized to Janus Green staining to adjust for cell plating differences.

Colony forming assays were accomplished from cells irradiated with gamma irradiation doses of 0-8 Gy, with and without 60 mM oxamate or 20 mM 2-Deoxy-D-glucose treatment. After irradiation, cells were seeded in culture dishes and incubated at 37°C/5% CO_2 for 8 days. Subsequently, colonies were stained with methylene blue (Merck KGaA, Darmstadt, Germany) and were quantified by computerized image analysis (ImageJ).

Double strand breaks were analyzed by counting the number of γH2AX foci per nucleus 1 and 24h after gamma irradiation with and without 60 mM oxamate or 20 mM 2-Deoxy-D-glucose treatment.

Results:

Both cell lines showed a significant decrease ($p < 0.001$) of ECAR and an increase of OCR after addition of oxamate (Figure 1 a + b). OC316 cells revealed a 62% reduction in ECAR and an increase to 168% in OCR. ECAR and OCR of IGROV-1 were decreased by 67% and increased to 149%, respectively. The injection of 2-Deoxy-D-glucose showed similar effects (Figure 1 b + c). ECAR of OC316 and IGROV-1 decreased 72% and 74%, respectively, and OCR increased 43% and 65%, respectively.

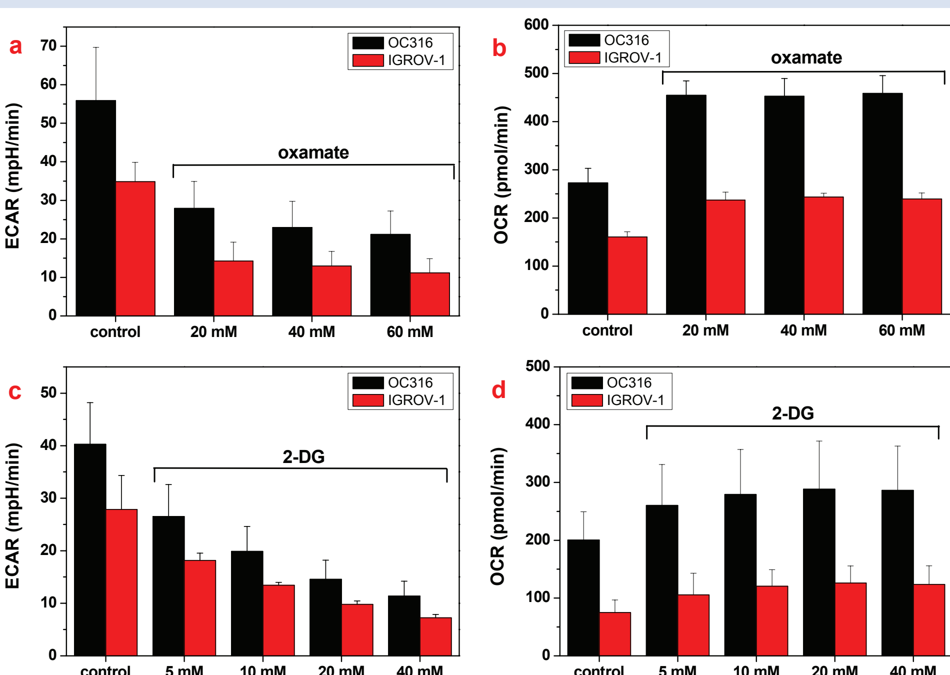

Figure 1: Extracellular acidification rates (ECAR) and oxygen consumption rates (OCR) of OC316 and IGOV-1 cells

a + b: Both cell lines show a significant decrease of ECAR and an increase of OCR after adding 20-60 mM oxamate (mean+sd; n=3).

c + d: ECAR decreased significantly and OCR increased after 5-40 mM 2-DG (2-Deoxy-D-glucose) injection (mean+sd; n=3).

Dose response curves illustrated a higher radiation resistance of untreated IGROV-1 cells compared to OC316 (Figure 2). Clonogenic cell survival increased up to 172% or 175% after 4 Gy X-ray irradiation with oxamate compared to control cells of OC316 and IGROV-1, respectively. OC316 cells revealed a 36% reduction of clonogenic cell survival after 2-Deoxy-D-glucose treatment in combination with 4 Gy irradiation. 8 Gy potentiated the oxamate and 2-DG effects.

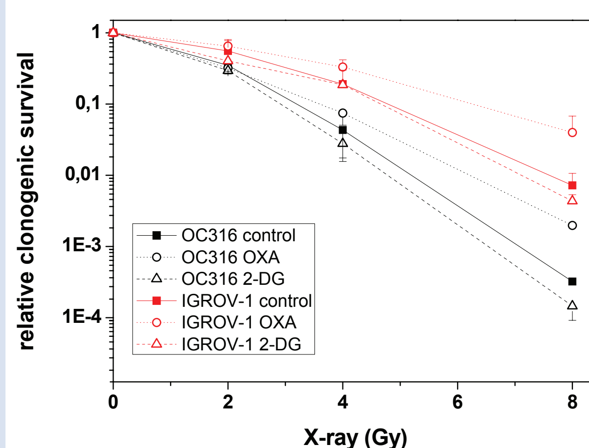

Figure 2: Relative clonogenic survival as a function of dose following gamma irradiation of OC316 and IGROV-1 cells.

Mean relative clonogenic survival after gamma irradiation untreated and treated with 60 mM OXA (oxamate) or 20 mM 2-DG (2-Deoxy-D-glucose). Each date point results from 3-6 independent experiments (±SD).

Dose response curves illustrate a higher radiation resisitance after gamma irradiation with oxamate and a sensitizing effect after 2-DG treatment compared to control cells of OC316 and IGOV-1.

For both cell lines the number of γH2AX foci was elevated dose- and time-dependently (Figure 3). Consistent with their higher radiation resistance, IGROV-1 showed an enhanced reduction of γH2AX foci 24h after irradiation. Oxamate decreased the formation of double strand breaks whereas 2-Deoxy-D-glucose caused a sensitizing effect for both cell lines.

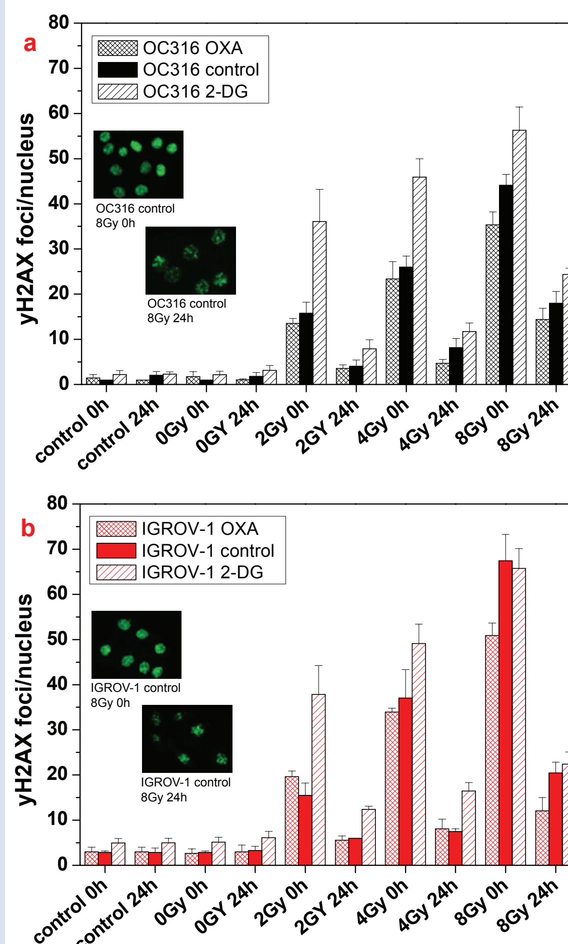

Figure 3: γH2AX foci per nucleus as a function of gamma irradiation dose and time

a: OC316 γH2AX foci per nucleus after gamma irradiation and treatment with 60 mM OXA (oxamate) or 20 mM 2DG (2-Deoxy-D-glucose) compared to control cells (mean+sd; n=3).

The formation of γH2AX foci is dose- and time-dependent. γH2AX foci/nucleus decrease 24h after gamma irradiation Oxamate treatment diminishes and 2-Deoxy-D-glucose raises the number of foci/nucleus.

b: IGROV-1 γH2AX foci per nucleus after gamma irradiation and treatment with 60 mM OXA (oxamate) or 20 mM 2DG (2-Deoxy-D-glucose) compared to control cells (mean+sd; n=3).

The maximum value is higher compared to OC316 cells. Principally oxamate decreases and 2-Deoxy-D-glucose increases the amount of foci/nucleus.

Conclusion:

- Oxamate and 2-Deoxy-D-glucose highly influence tumor glycolysis.
- Oxamate, a pyruvate analog and LDH inhibitor seems to act as an irradiation protective reagent.
- The competitive inhibitor of glucose metabolism, 2-Deoxy-D-glucose, shows a radiation sensitizing effect.
- The data support the hypothesis that targeting tumor glycolysis could influence the effects of irradiation therapy.

References:

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