Metabolic plasticity and adaptive resistance to metabolic inhibitors in tumor cells

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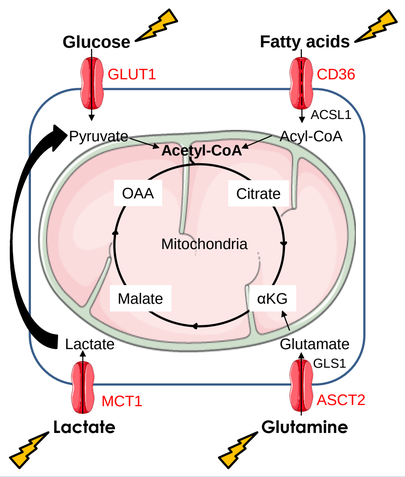
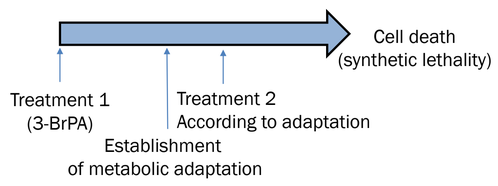
INTRODUCTION

Nowadays, tumor metabolism represents an attractive field to develop new anticancer drugs. However, tumor metabolic plasticity is such that cancer cells can adapt their metabolic preferences according to the (un-)availability of nutrients or the (in-)capacity to transform them into biosynthetic intermediates and ATP. Direct consequences of metabolic adaptation are the development of resistance and the progressive loss of activity of a metabolism-targeting drug, thereby limiting the clinical applicability of such compounds as monotherapies.

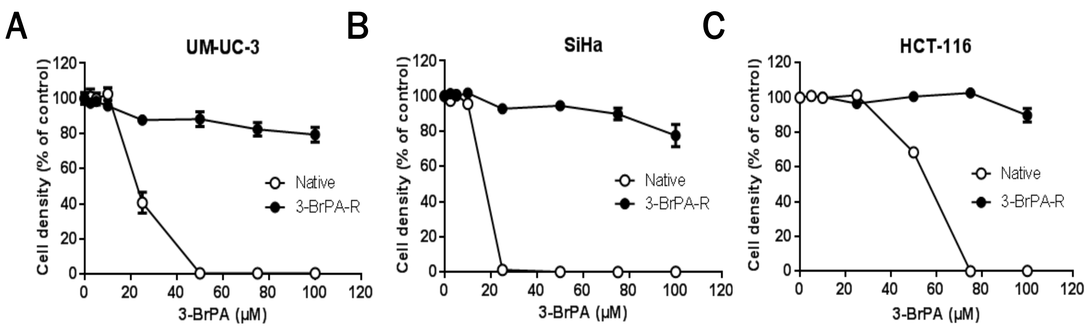


Aim: To identify the optimal combination of metabolism-targeting drugs, in order to prevent a possible escape for cancer cells. Our rationale is, with a first drug, to force cancer cells to become addicted to specific bioenergetic fuels/pathways in order to render a second drug lethal.



Proof of principle: 3-bromopyruvate (3-BrPA) as a first metabolism-targeting drug to generate resistant clones in three different cancer cell lines: UM-UC-3 (bladder carcinoma), SiHa (cervix carcinoma) and HCT-116 (colorectal carcinoma).

1. Establishment of 3-BrPA-resistant tumor cells



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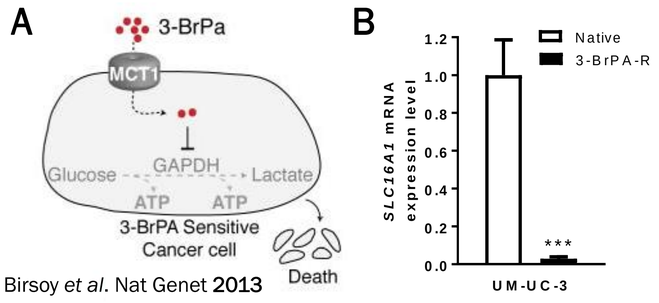
Cell lines		IC_{50} (µM) ± SD
UM-UC-3	Native	24.18 ± 2.057
	3-BrPA-R	> 100
SiHa	Native	14.67 ± 0.353
	3-BrPA-R	> 100
HCT-116	Native	28.60 ± 1.575
	3-BrPA-R	> 100

(**A-C**) Cell growth of native and 3-BrPA-R UM-UC-3 (**A**), SiHa (**B**) and HCT-116 (**C**) cancer cells following treatment with increasing 3-BrPA concentrations for 72h. (**D**) IC_{50} values (µM) for 3-BrPA in native and 3-BrPA-R UM-UC-3, SiHa and HCT-116 cancer cells.

2. Epigenetic MCT1 silencing as a source of resistance to 3-BrPA



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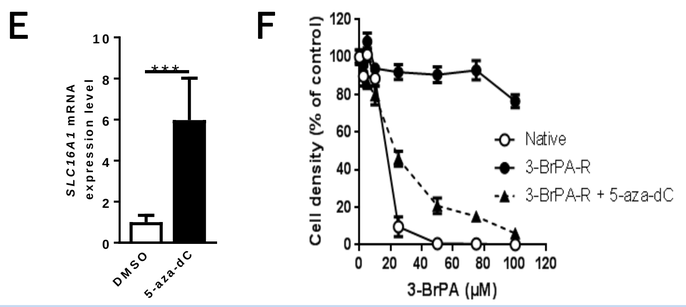
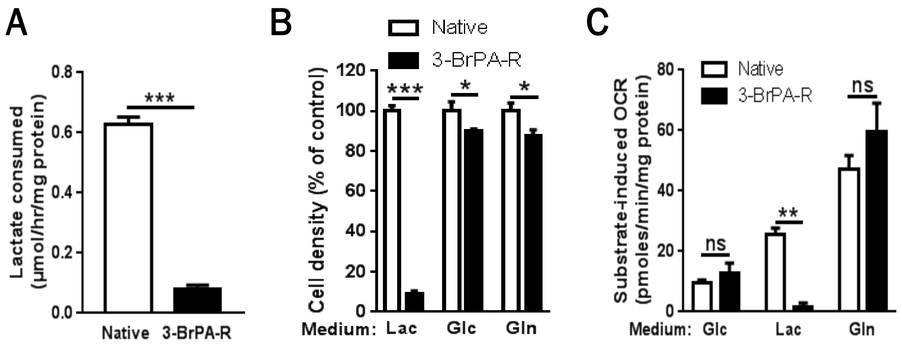


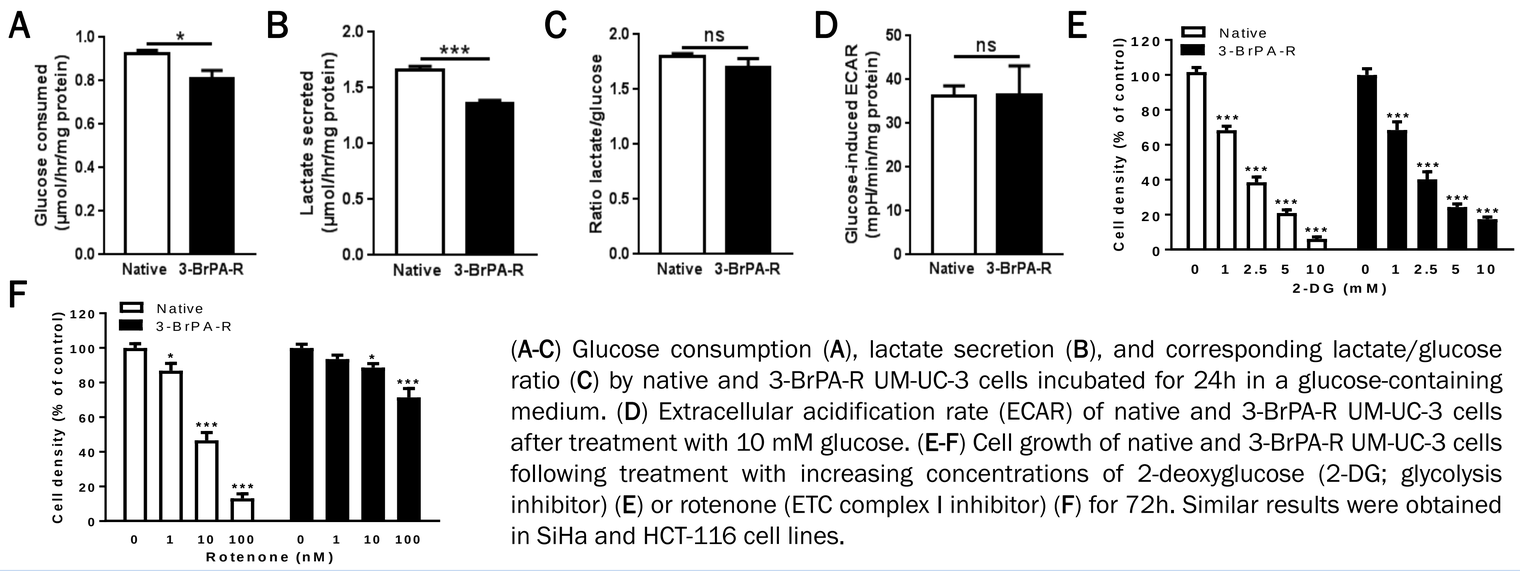
(**A**) Scheme depicting monocarboxylate transporter 1 (MCT1) as the main entry path for 3-BrPA. (**B-C**) mRNA (**B**) and protein (**C**) expression of MCT1 in native and 3-BrPA-R UM-UC-3 cells. (**D**) Representative immunofluorescence pictures for MCT1 in native and 3-BrPA-R UM-UC-3 cells; scale bar:10 µm. (**E-F**) *SLC16A1* (MCT1) mRNA expression (**E**) and cell growth for 72h in 3-BrPA-containing medium (**F**) for 3-BrPA-R UM-UC-3 cells following treatment with the demethylating agent 5-azadeoxycytidine (5-aza-dC). Similar results were obtained in SiHa and HCT-116 cell lines.

3. Lactate uptake and utilization are impaired in 3-BrPA-R tumor cells



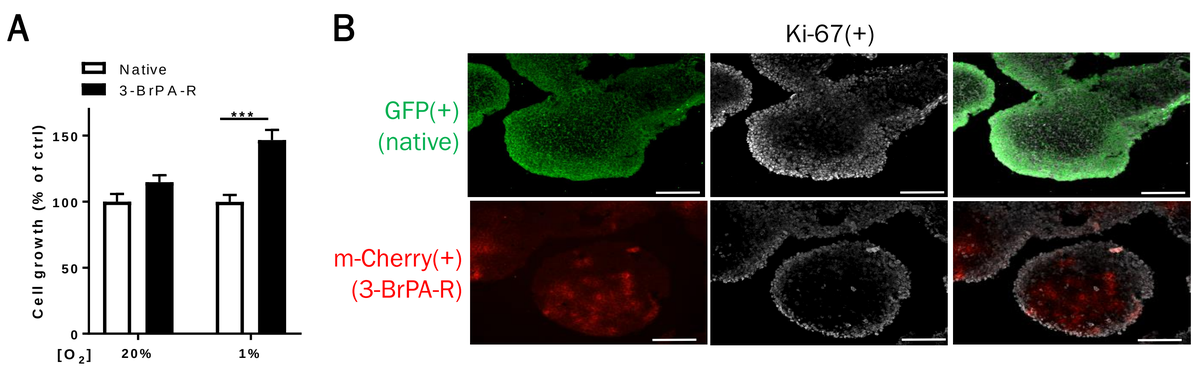
(**A**) Lactate consumption by native and 3-BrPA-R UM-UC-3 cells incubated for 24h in a lactate-containing medium. (**B-C**) Cell growth (72h) (**B**) and oxygen consumption rate (OCR) (**C**) of native and 3-BrPA-R UM-UC-3 cells in lactate-, glucose- or glutamine-containing media. Similar results were obtained in SiHa and HCT-116 cell lines.

4. Glycolytic flux is maintained and preferred to OXPHOS in 3-BrPA-R cells



(**A-C**) Glucose consumption (**A**), lactate secretion (**B**), and corresponding lactate/glucose ratio (**C**) by native and 3-BrPA-R UM-UC-3 cells incubated for 24h in a glucose-containing medium. (**D**) Extracellular acidification rate (ECAR) of native and 3-BrPA-R UM-UC-3 cells after treatment with 10 mM glucose. (**E-F**) Cell growth of native and 3-BrPA-R UM-UC-3 cells following treatment with increasing concentrations of 2-deoxyglucose (2-DG; glycolysis inhibitor) (**E**) or rotenone (ETC complex I inhibitor) (**F**) for 72h. Similar results were obtained in SiHa and HCT-116 cell lines.

5. Glycolytic 3-BrPA-R tumor cells are particularly suited to survive under hypoxia



(**A**) Cell growth of native and 3-BrPA-R UM-UC-3 cells incubated under normoxic (20% O_2) or hypoxic (1% O_2) conditions for 48h. (**B**) Representative immunofluorescence pictures for GFP, m-Cherry and Ki-67 (proliferation marker) stainings in 3D mixed spheroids, composed of GFP- and mCherry-labelled native and 3-BrPA-R HCT-116 cells, respectively; scale bar: 200 µm. (**C**) Light-sheet microscopy imaging of a CUBIC-cleared mixed 3D spheroid composed of GFP- and mCherry-labelled native and 3-BrPA-R HCT-116 cells, respectively; scale bar: 200 µm.

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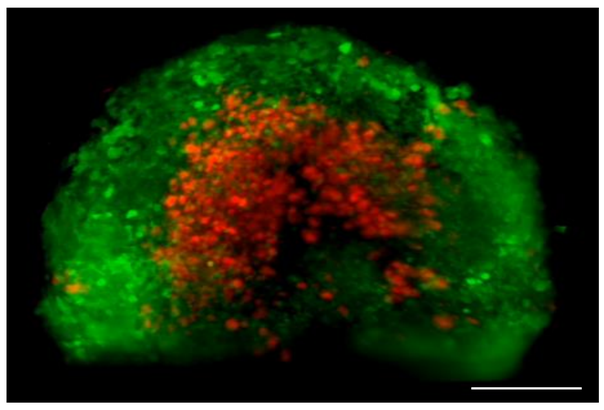
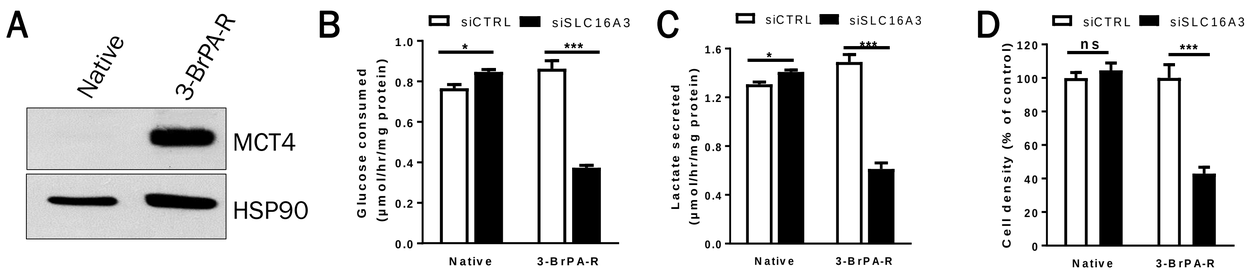
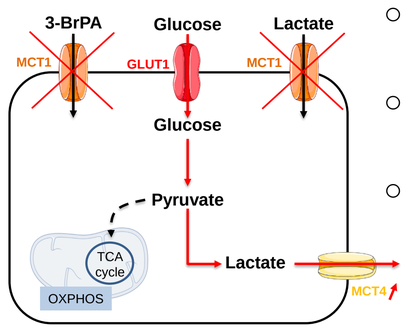


6. MCT4 supports glycolysis and growth in 3-BrPA-R tumor cells



(**A**) MCT4 protein expression in native and 3-BrPA-R UM-UC-3 cells. (**B-C**) Glucose consumption (**B**) and lactate secretion (**C**) by native and 3-BrPA-R UM-UC-3 cells incubated for 24h in a glucose-containing medium, following transfection with control or MCT4- (*SLC16A3*) targeting siRNA. (**D**) Cell growth of siCTRL- and siMCT4-transfected native and 3-BrPA-R UM-UC-3 cells for 72h. Similar results were obtained in SiHa and HCT-116 cell lines.

CONCLUSIONS AND PERSPECTIVES



- 3-bromopyruvate resistance results from an epigenetic downregulation of MCT1 gene;
- 3-BrPA-R tumor cells exhibit a glycolytic phenotype adapted to survival under hypoxia and highly dependent on MCT4 expression;
- Metabolic adaptation to 3BrPA can be therapeutically targeted via MCT4 silencing (or pharmacological blockade), thereby highlighting the sequential administration of metabolic inhibitors as an agttractive strategy to overcome metabolic plasticity and associated therapy resistance