SCIENTIFIC REPORTS
nature research

Glycolytic suppression dramatically changes the intracellular metabolic profile of multiple cancer cell lines in a mitochondrial metabolism-dependent manner

Reika Shiratori[1], Kenta Furuichi[1], Masashi Yamaguchi[2], Natsumi Miyazaki[1], Haruna Aoki[1], Hiroji Chibana[2], Kousei Ito[1] & Shigeki Aoki[1*]

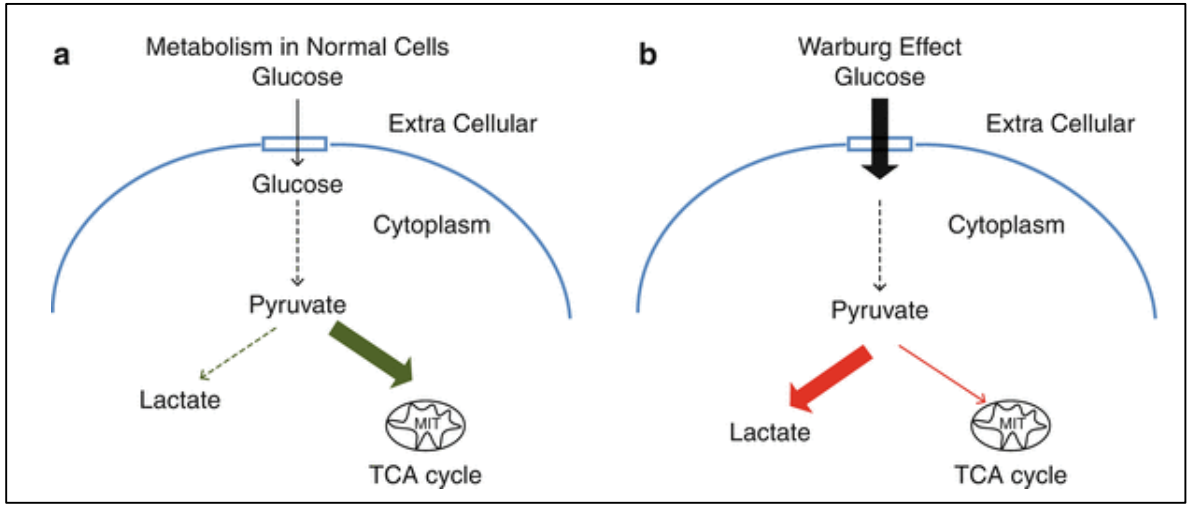
Journal Club
Max Holzknecht
04.06.2020

Content

- Introduction:
  - Energetic remodeling in cancer cells
- Experimental Setup
- Results
- Outcome
- Discussion

Energetic remodeling in cancer cells

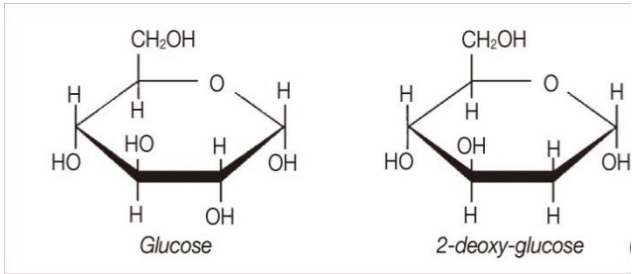
- Cancer cells obtain their energy by increasing **anaerobe glycolysis** *(Warburg effect)*
- Glucose is converted through glycolysis to pyruvate and finally lactate



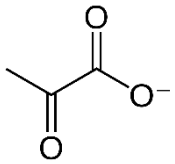
- no vessel formation in fast proliferating tumors → no oxygen available → secretion of lactate and **acidification** of the tumors **micro-environment** is a typical metabolic signature of cancer cells
- Additionally, glutaminolysis, which supports **biosynthesis** and maintains **bioenergetics** and redox balance, is critical for cancer cell growth

Targeting cancer cells: relevance in chemotherapy

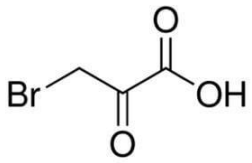
- The energetic remodeling towards glycolysis is a suitable target for therapies:
  - GLUT1: Glucose transporter
  - Hexokinase
  - LDHA (Lactate dehydrogenase A)



- Molecular analogs:
  - 2-deoxyglucose (inhibits G-6-P-Isomerase)
  - 3-bromo-pyruvate (inhibits GAPDH)



pyruvate



3-Bromo-pyruvate

- A **combination therapy** using a glycolytic inhibitor with other anti-cancer drugs is more effective at suppressing tumors
- Cancer cells can **adapt** their metabolism to survive when glycolysis is suppressed, and it is necessary to understand how cancer cells **modulate** their intracellular **metabolism** under these conditions

Experimental Setup

Cancer cell lines:

- **PANC-1** pancreatic cancer cells
- and two other solid tumor cell lines **A549** and **HeLa**

➢ Cultured in **glucose/low glucose or galactose** containing media

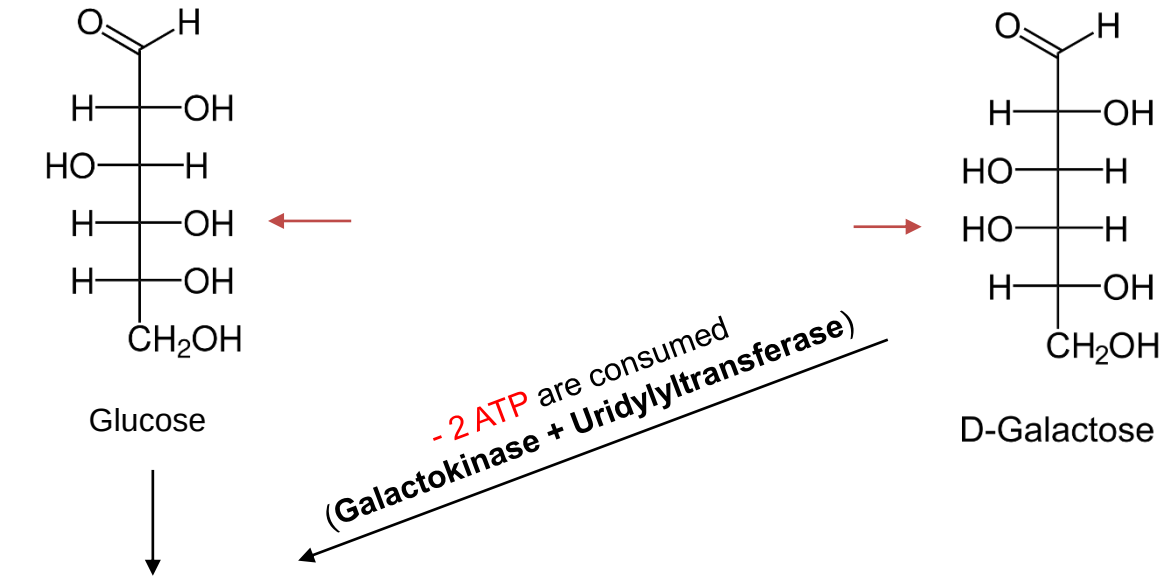
Metabolomics:

- CE-TOFMS (Capillary Electrophoresis Time Of Flight **Mass Spectrometer**)

Biological analysis:

- Flow cytometry
- Fluorescence microscopy
- Electron microscopy
- Viability assays

Why is galactose used to test cells for mitochondrial function?



- **Galactose** as main substrate:
  - ⇩ Glycolysis
  - ⇧ <u>OXPHOS</u>
- **Glucose** as main substrate:
  - ⇳ Glycolysis
  - ⇳ OXPHOS

Results

SCIENTIFIC REPORTS
nature research

Glycolytic suppression dramatically changes the intracellular metabolic profile of multiple cancer cell lines in a mitochondrial metabolism-dependent manner

Reika Shiratori[1], Kenta Furuichi[1], Masashi Yamaguchi[2], Natsumi Miyazaki[1], Haruna Aoki[1], Hiroji Chibana[2], Kousei Ito[1] & Shigeki Aoki[1*]

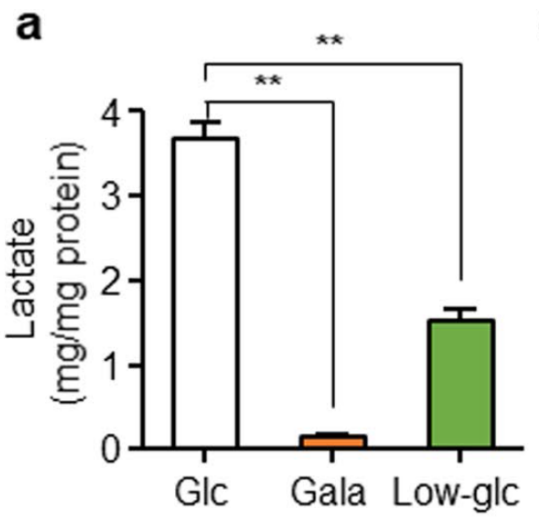
Results

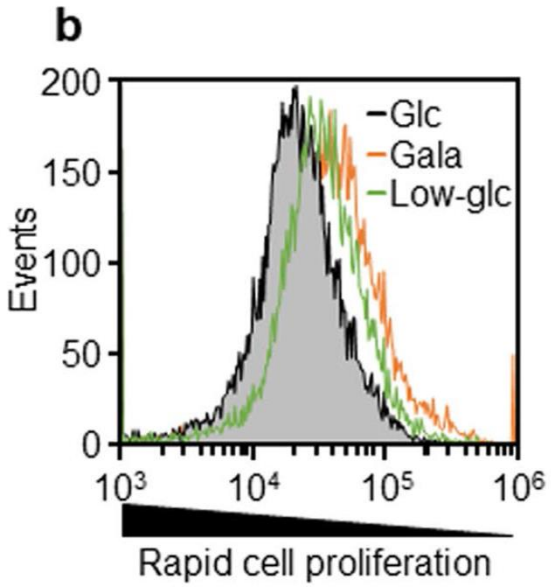
Glycolytic suppression dynamically changes the glycometabolism profile of PANC-1 cells

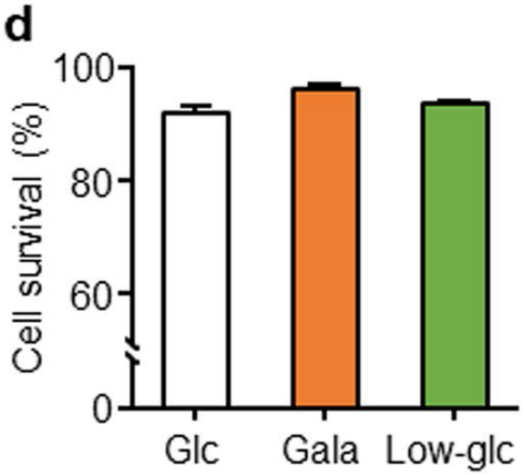
- **Lactate secretion** is strongly decreased → suppression of glycolysis in presence of galactose/low glucose

- cell proliferation was monitored *via* carboxyfluorescein diacetate succinimidyl ester (CFSE)
- Proliferating cells show less staining → dye is reduced in the daughter cells

- Suppressing glycolysis did not affect cell survival, although their growth was mildly suppressed





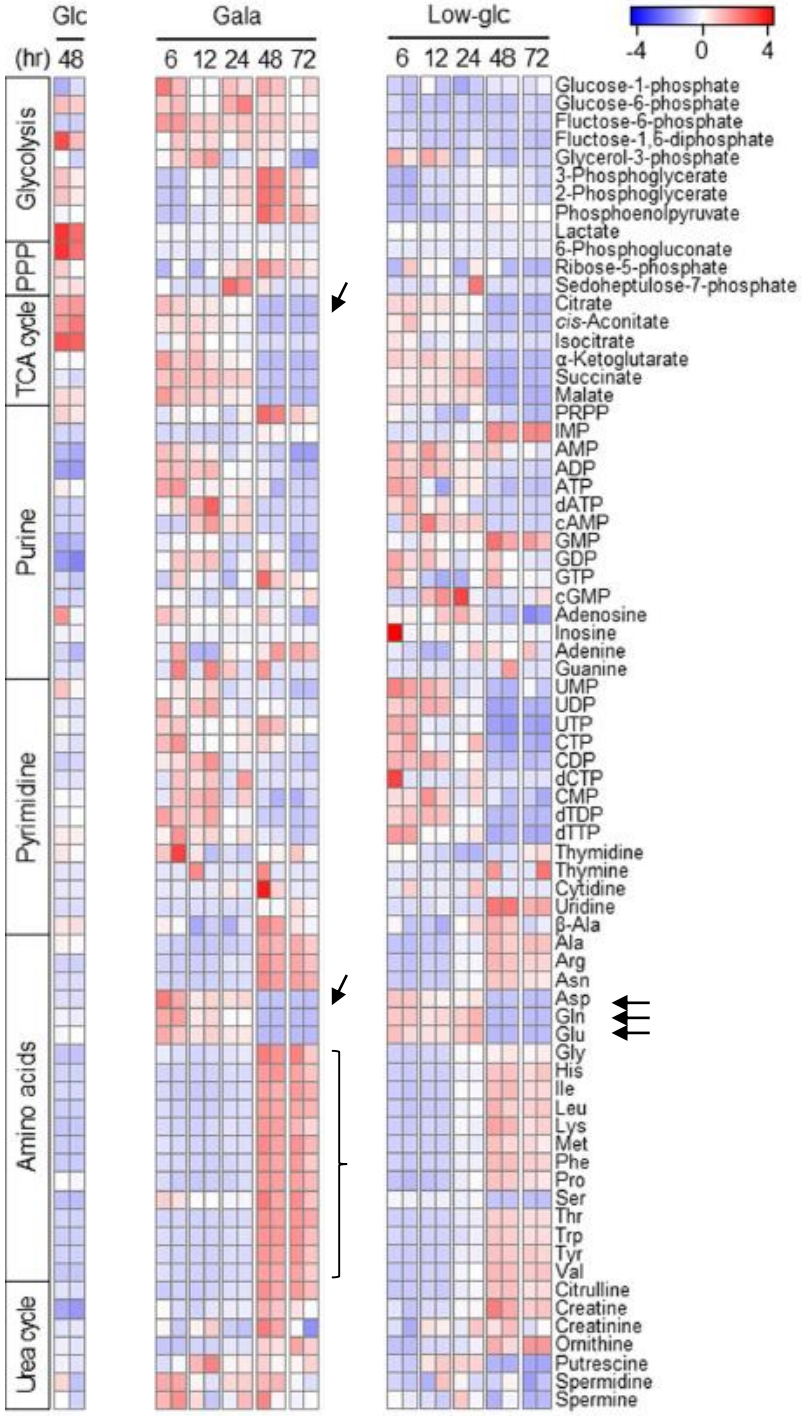


Results

Glycolytic suppression dynamically changes the glycometabolism profile of PANC-1 cells

- **CE-TOFMS: quantification of water soluble metabolites**
  - glycolytic products
  - intermediates of the TCA cycle
  - amino acids
  - …
- Dramatic reduction of **lactate** levels
- Intermediates of **TCA cycle** were reduced
- Levels of **amino acids** were increased
- **Glutamine, glutamate and aspartate** were decreased
- TCA cycle enzymes were increased upon suppression of glycolysis (supp. Data)

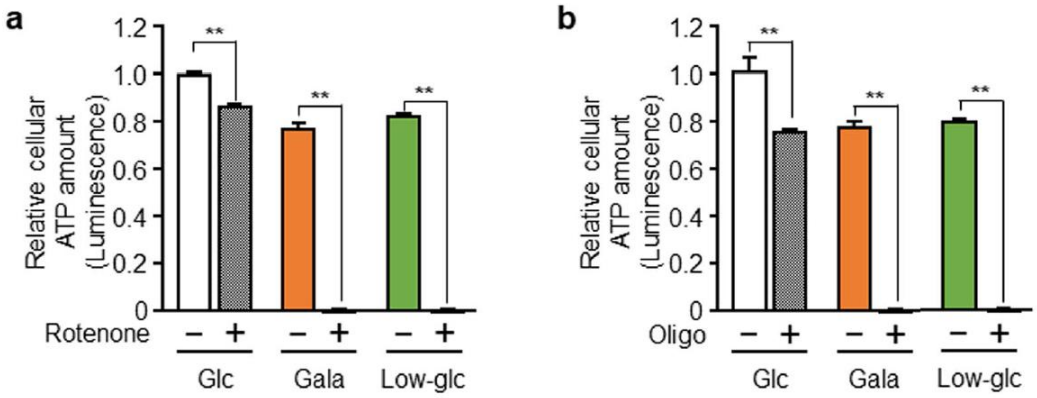
Cancer cells shift their metabolism towards OXPHOS and AA utilization when glucose is not available

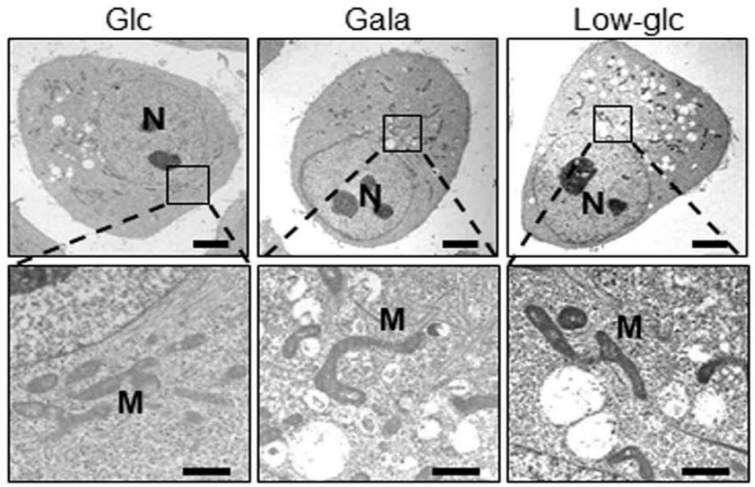


Results

Upregulation of mitochondrial function results in enhanced survival of PANC-1 cells

- in mammalian cells, ATP is obtained *via* **glycolysis and/or OXPHOS**
- Inhibition of glycolysis **in combination** with inhibition of OXPHOS results in strongly decreased ATP levels
- Cells cultured in galactose or low glucose containing media are highly sensitive for both
  - rotenone: inhibits complex I
  - oligomycine: inhibits complex V (ATP-synthase)
- Mitochondrial morphology was shifted towards “fusion” in glycolysis suppressed cells





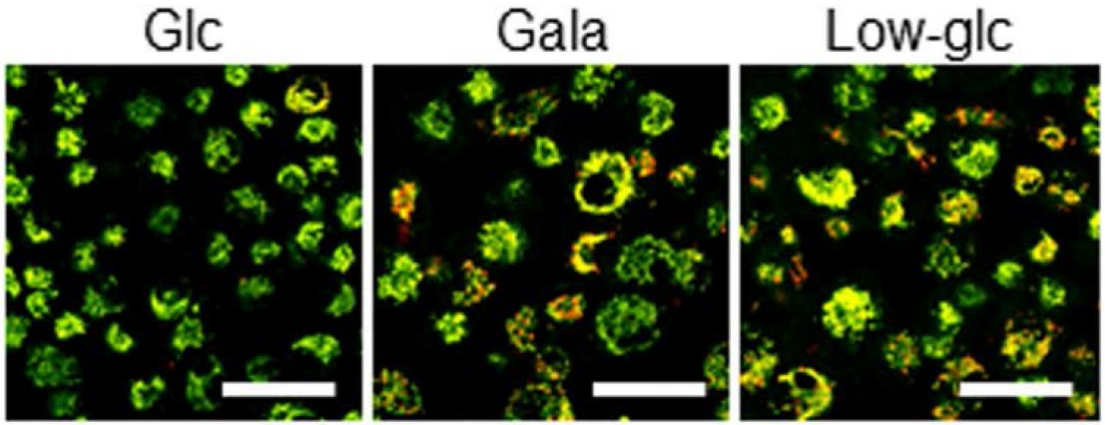
Transmission electron microscopy

OXPHOS activity is necessary to maintain cellular ATP levels and to support survival

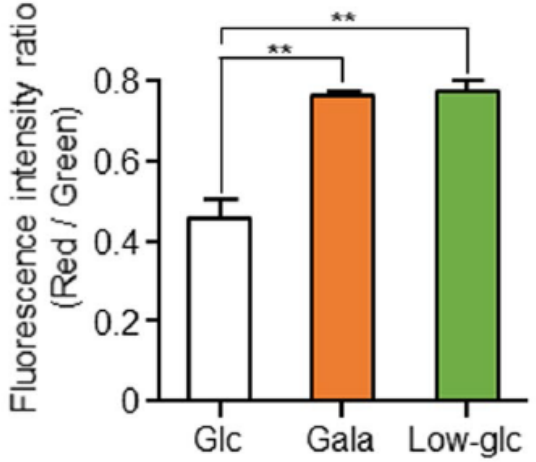
Results

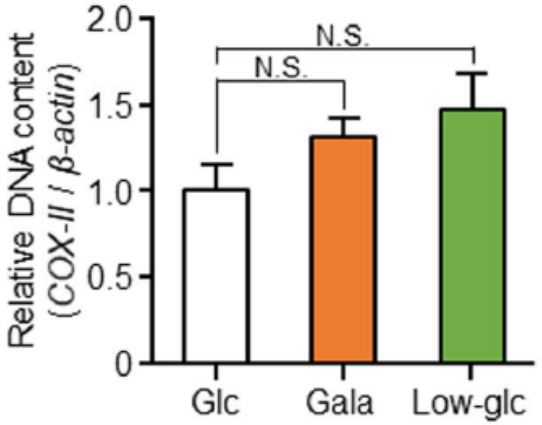
Glycolytic suppressed cells have a higher mitochondrial functionality

- Accumulation of JC-1 leads to polymerization and indicates higher uptake
  → higher membrane potential
- MitoTracker Orange was used to confirm these findings (supp. data)
- Mitochondrial DNA content was increased (*cytochrome c oxidase subunit II*)
- As a control, 2-deoxyglucose was used to suppress glycolysis
  → co-treatment with oligomycin led to sign. decreased ATP levels (supp. data)



JC-1: high mitochondrial membrane potential (yellow/red)



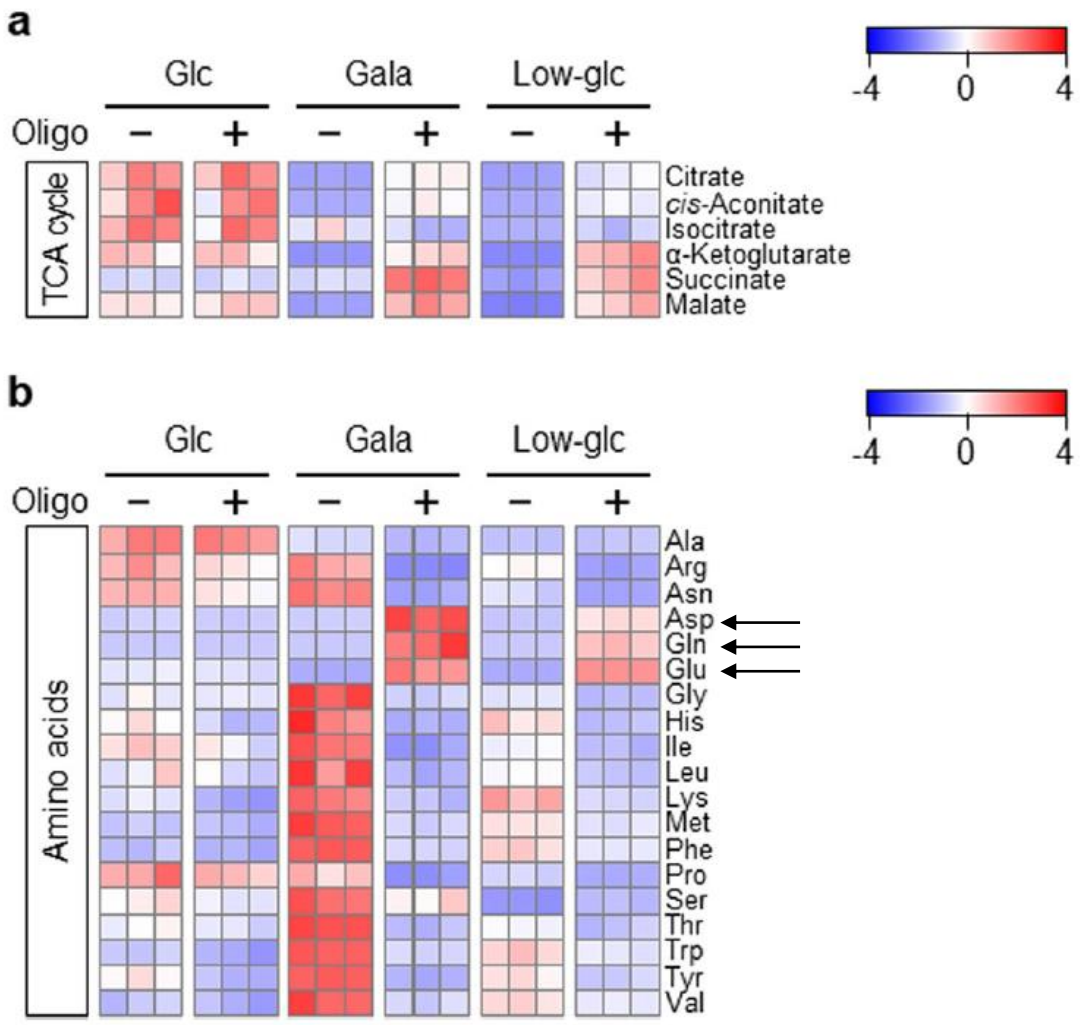


Mitochondrial quality was increased in PANC-1 cells whereas mitoch. mass was hardly affected

Results

Intracellular energy metabolism is reprogrammed towards mitochondrial OXPHOS

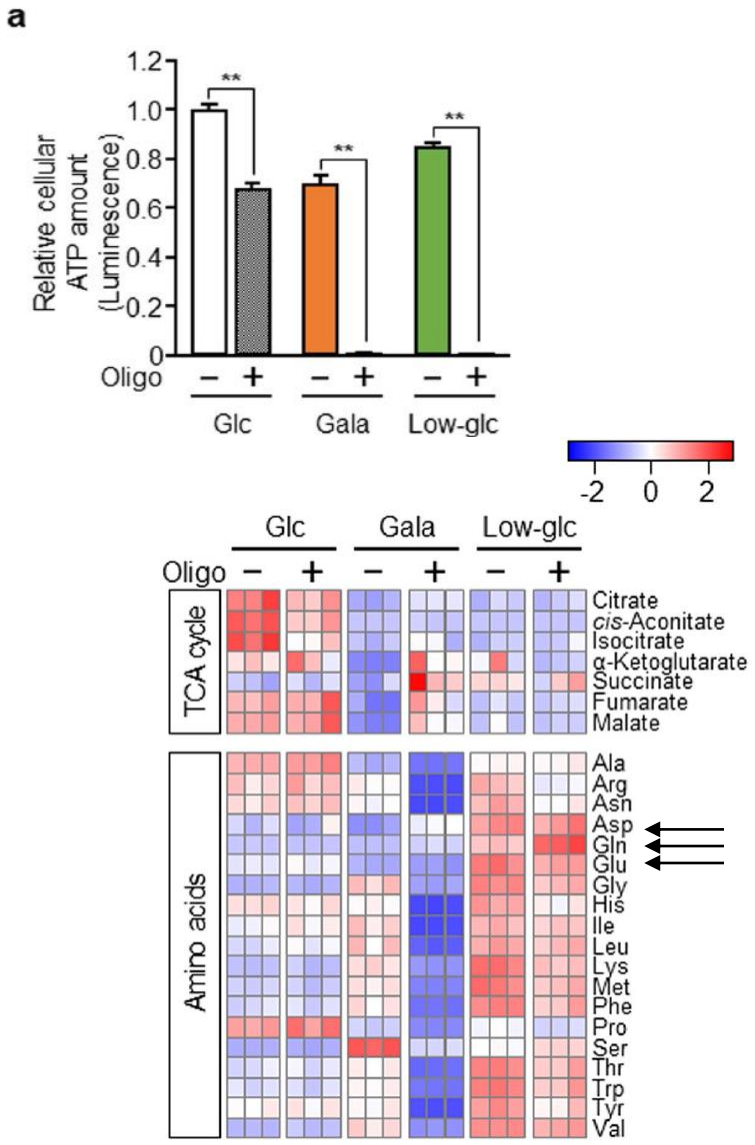
- **oligomycin** (0.8 ng/ml) was used to **reverse** the changes in the metabolic profile of PANC-1 cells
- the decrease in TCA cycle intermediates was reversed through the treatment with oligomycin
- amino acid levels are reduced due to OXPHOS inhibition
- **glutamine, glutamate and aspartate** levels were affected and show the **opposite pattern** compared to other amino acids



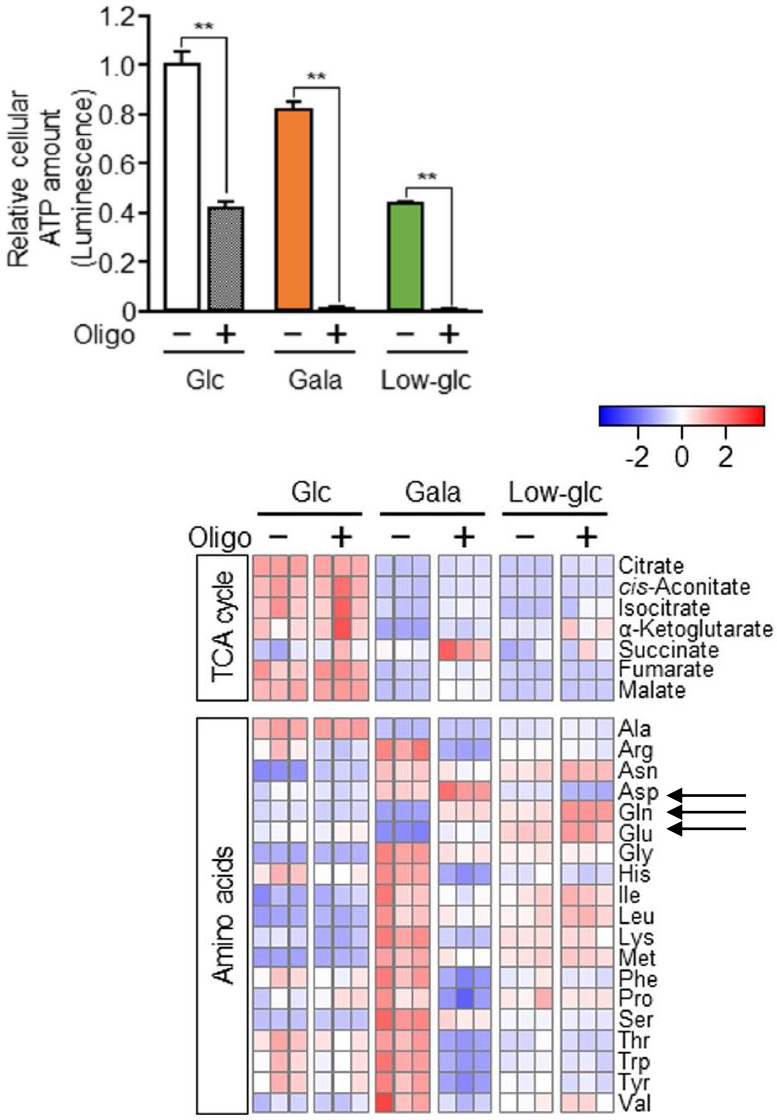
Dynamic changes in intracellular amino acid levels in glycolysis suppressed cells are closely related to the induction of OXPHOS

Metabolic reprogramming also occurs in other cancer cells

A549: Adeno-carcinoma (lung)



HeLa: cervical cancer

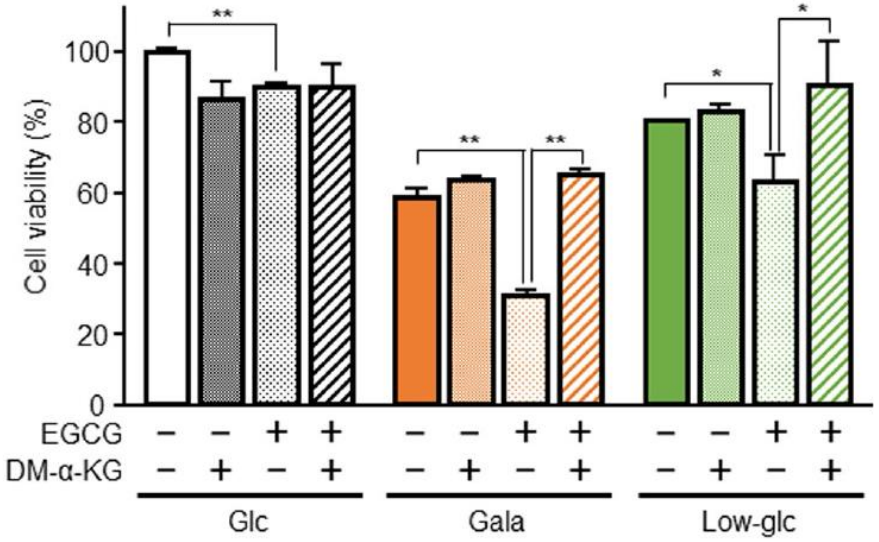


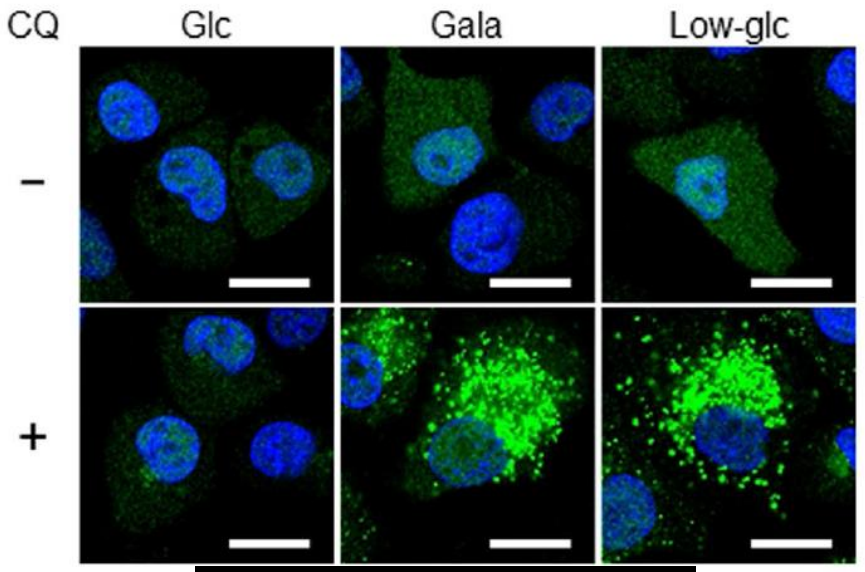
Results

Autophagy mediates metabolic reprogramming and is needed to supply the mitochondria with metabolites

- Cells were treated with a glutaminolysis inhibitor which inhibits GDH1 (glutamate-dehydrogenase I)
- Di-methyl-a-ketoglutarate rescued the reduction of cellular viability
- Influx of glutamine and glutamate into the TCA cycle drives mitochondrial activity in glycolytic suppressed cells
- Autophagic flux is strongly increased → degradation of proteins and organelles to fuel the TCA cycle with Glu/Gln

MTT assay: cell viability





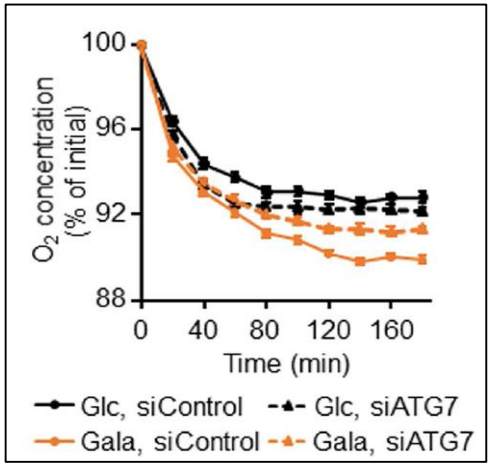
Autophagy: **LC3-GFP** and **TO-PRO-3**

Autophagy is upregulated in glycolytic suppressed cells

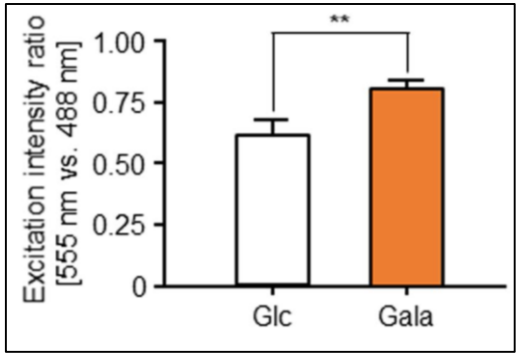
Results

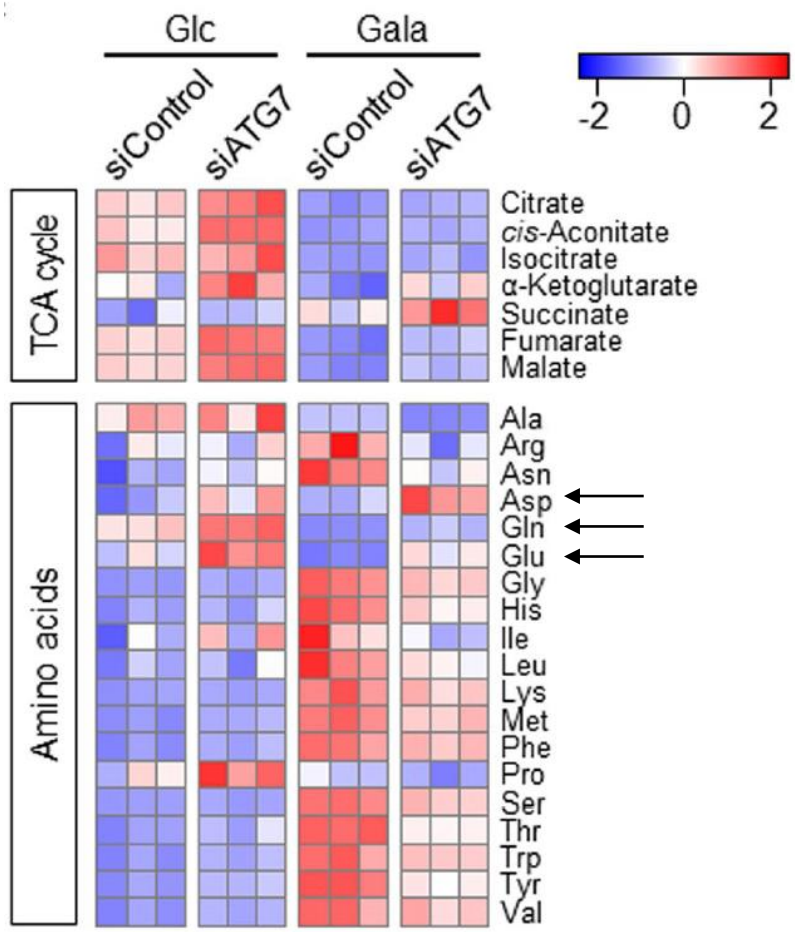
Knock-down of ATG7 reduces usage of amino acids

- *Autophagy related* (*ATG7*) gene was knocked down *via* RNA interference
- Amino acid levels are increased under control conditions (siControl) if glycolysis is suppressed
- Reductions of TCA intermediates levels and the elevations in the levels of amino acids tended to be reversed by ATG7 knock-down
- Autophagy seems to be necessary for the supply with amino acids in glycolytic suppressed cells



mtKeima-Red:
mitochondria in acidic environment

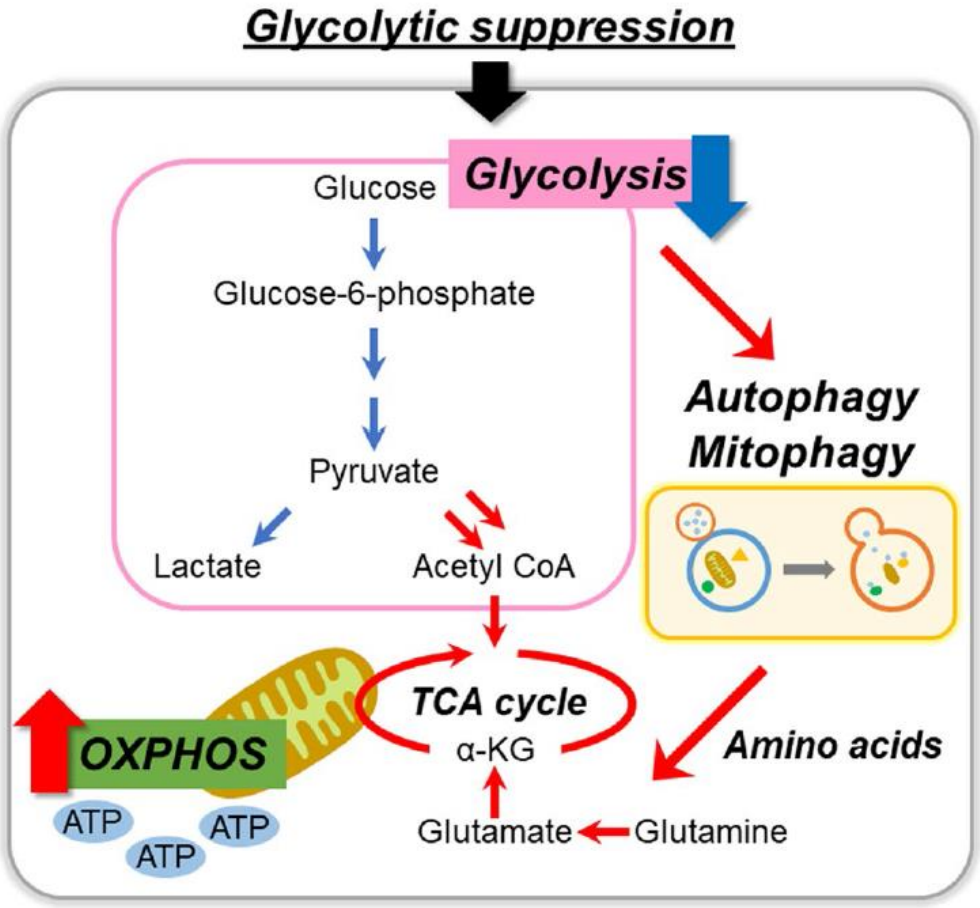




Inhibition of autophagy (including **mitophagy**) reduces the supplies with amino acids that are necessary for driving mitochondrial OXPHOS

Outcome

Regulation of the energy metabolism in glycolytic suppressed cancer cells



Discussion