Systematic approaches to study cancer cell metabolism

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Cellular metabolism is complex
Metabolic pathways contribute to cellular functions. Cells require metabolic rewiring to proliferate.

Metabolic Pathways

- Energy
- Building blocks
- Post-translational Modifications
- Signaling

Cellular Functions

- Fatty acids
- Cholesterol
- Nucleic acids
- Proliferation
- Differentiation
- Response to the environment
Understand which metabolic pathways are required for cancer cell proliferation

Determine the metabolic diversity among cancer cells
Cancer cells have different metabolism than normal cells

Differentiated Cells

Proliferating Cells
Most cancer cells respire
Cells require electron transport chain activity for proliferation.

Fatty acids, cholesterol, nucleic acids, glucose, amino acids, vitamins, ATP, CO\textsubscript{2}, O\textsubscript{2}, ETC inhibitors.
The mitochondrial electron transport chain is involved in multiple biological processes.
Functional Genomics Screens in mammalian cells

shRNA based screens

Birsoy et. al., Nature (2014)
A CRISPR based genetic screen
synthetic interactions with phenformin

30,000 sgRNAs
~3000 metabolic genes

sgRNA library construction

Jurkat Cell Line

Pool Infection

Pool of Knock out cells

UNTREATED

PHENFORMIN

Compare sgRNA abundance
Metabolic genes that enable growth under ETC inhibition

Aspartate \[\rightarrow\] GOT1 \[\rightarrow\] OAA
a-KG \[\rightarrow\] PLP \[\rightarrow\] Glutamate

PDXK

Pyridoxal

Differential gene score
(Phenformin-Utreated)

-1.5 -1.0 -0.5 0.0

NDUFA11
CAD
NDUFB2
DAD1
COQ4
GSS
SCO2
GPI
SLC25A1
PMM2
MCAT
GU51
UROD
PDE12
ADSL
ACAD9
CTPS1
TRPC1
ACADL
NDUFA10
ATP8B3
NDUFB9
TYMS

Red: GOT1 related
Blue: ETC
Green: Nucleotide syn.
Black: not categorized
ETC inhibition kills cells lacking GOT1
GOT reaction reverses under mitochondrial respiration inhibition

Wild Type

GOT1 null

Protein synthesis

Purine and Pyrimidine synthesis

Cellular Aspartate levels

Relative abundance compared to control

MITOCHONDRIA

CYTOSOL
Aspartate addition enables cell proliferation and viability under ETC inhibition.

Aspartate addition enables cell proliferation and viability under ETC inhibition.

**Aspartate: 10 mM**
Pyruvate supplementation can rescue proliferation defects under ETC inhibition.
Pyruvate supplementation provides NAD⁺ under ETC inhibition

[27] Isolation of Human Cell Lines Lacking Mitochondrial DNA

By Michael P. King and Giuseppe Attardi

The reduction of pyruvate to lactate through the activity of the NAD-linked lactate dehydrogenase could provide a means for oxidizing the excess cytoplasmic NADH. This reduces the amount of pyruvate that...
Pyruvate-mediated rescue of ETC inhibition is dependent on GOT1

Pyruvate $\rightarrow$ **GOT1** $\rightarrow$ Aspartate $\rightarrow$ **ETC inhibitors**

| Phenformin: | - | + | + | - | + | + |
| Pyruvate:   | - | - | + | - | - | + |
| Jurkat clones: | Wild Type | GOT1_KO2 |

Fold change in cell number in 5 days (log2)

- ** - ns

*Data presented as mean ± SEM.*

**P < 0.01

Relative aspartate abundance

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**GOT1:** Glutamate Oxaloacetate Transaminase 1
Studying mtDNA mutations in culture

EtBr treatment → Fusion

How do we grow these cells with mtDNA mutations?
Aspartate is sufficient to enable proliferation of cybrids with patient-derived mtDNA mutations
Strategies to increase aspartate in the serum

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>&lt;1 month</th>
<th>1-23 months</th>
<th>2-17 years</th>
<th>Adults (≥18 years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic acid</td>
<td>2-20</td>
<td>2-14</td>
<td>1-8</td>
<td>1-4</td>
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</tbody>
</table>

Aspartate used in our experiments: 10 mM
Aspartate is sufficient to enable proliferation of cybrids with patient-derived mtDNA mutations.
Aspartate levels decrease upon prolonged hypoxia
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