Regulation of cell metabolism

Metabolic networks are dynamic
Catabolism and Anabolism determine the metabolic status of the cell.
Supply and demands dictate metabolism

Diagram showing the relationship between producers, pool of metabolites, and consumers, with metabolic controls regulating the flow of supply and demand.
Supply and demands dictate metabolism

1. METABOLIC DEMANDS
   • Growth and proliferation
   • Differentiation

2. METABOLIC SUPPLY
   • Tissue specificity
   • Hormonal stimulation
   • Diet
   • Perfusion
Proliferation presents metabolic challenges

**Quiescent cell**
- Glucose
- Fatty acids
- Glycolysis
- ATP
- TCA cycle
- Glutamine

**Proliferating cell**
- Glucose
- To biomass
- ATP
- CO₂
- Glutamine

**Enzyme**
- ATP
- Acids
- Fatty
- Glucose
- Glycolysis
- Glutamine
- Glutaminolysis
- To biomass
- Acetyl-CoA
- succinate
- Oxaloacetate
- Fumarate
- NADH
- NAD⁺
- Succinyl-CoA
- Oxaloacetate
- NADH
- NAD⁺

**Pathways**
- **Glycolysis**
- **Glutaminolysis**

**Cells**
- Quiescent
- Proliferating

**Conditions**
- Growth factor activation
- PI3K-Akt signaling
- mTOR
- p38
- JNK
- ERK

**Molecules**
- Glutamate
- Lactate
- Thr
- Serine
- Glutamine
- αKG
- f2HG
- PGAM1
- THF
- FAS
- PFKFB3
- IDH1/R2

**Pathway**
- Glucose
- Fatty acids
- ATP
- TCA cycle
- Glutamine

**Regulation**
- Akt
- JNK
- ERK
- MYC
- p53
- TET2

**Gene Expression**
- PHGDH
- PGAM1
- IDH1
- IDH2
- SDH

**Proteins**
- IDH1
- IDH2
- SDH

**Metabolites**
- Glucose-6-phosphate
- Fructose-6-phosphate
- Phosphoenolpyruvate
- 3-phosphoglycerate
- Pyruvate
- Oxaloacetate

**Cancers**
- Breast
- Prostate
- Colon
- Ovary
- Thyroid

**Mutations**
- Germline loss-of-function mutations in any of the four subunits of SDH
- IDH2 missense mutations at R172
- IDH1 somatic missense mutations at R132

**Cytoskeleton**
- Actin
- Myosin

**Cellular Stress**
- Cell stress
- Tumor suppression

**Signaling**
- Functional outcome
- Oncogenic alteration

**Pathogenic Events**
- Fumarate
- TCA cycle
- αKG
- 2HG

**Cellular Localization**
- NADH
- NAD⁺

**Regulatory Proteins**
- TET2
- Jumonji C histone demethylase enzymes

**Cell Survival**
- Cell proliferation
- Cell death

**Growth Factors**
- Growth factor
- PI3K
- Akt
- mTOR

**DNA Methylation**
- 5-methylcytosine hydroxylase TET2
- 5-methylcytosine

**Biochemical Pathways**
- Glycogen phosphorylase
- Glucose-6-phosphate dehydrogenase
- PFK2
- PFKFB3
- PKM2

**Cellular Metabolism**
- Glycolysis
- TCA cycle
- Glutaminolysis

**Cellular Processes**
- nongenomic lipogenesis
- Lipid synthesis

**Cancer Biology and Genetics Program**
- Memorial Sloan-Kettering Cancer Center, New York, NY 10065, USA

**Authors**
- Lydia W. S. Finley, Ji Zhang, Jiangbin Ye, Patrick S. Ward, and Craig B. Thompson

**References**
- Finley LY, Thompson CB, Cell, 2012

**DOI**
- http://dx.doi.org/10.1016/j.cmet.2013.02.016
Proliferation presents metabolic challenges

- **Finley LY, Thompson CB, Cell, 2012**

**Pathways SnapShot: Cancer Metabolism**

**FH** Hereditary mutations can underlie cutaneous and uterine leiomyomata. SDH Germline loss-of-function mutations in any of the four subunits of SDH can cause a variety of diseases, including hamartomas, pheochromocytomas, and renal cell cancer. IDH2 Missense mutations at R172 (the analogous residue to IDH1 R132) are prevalent in glioma, whereas IDH1 somatic missense mutations at R132 are prevalent in glioma, pancreatic carcinoma, and renal cell cancer. PKM2 Many cancer cells express the M2 isoform of PK. Tyrosine kinase inhibitors can selectively inhibit the kinase activity of PKM2 in cancer cells. PGAM1 PGAM1 is upregulated in colorectal cancer and hepatocellular carcinoma.

**PHGDH** Genomic regions containing the PHGDH gene are amplified in melanoma and renal cell cancer. PFKFB3 The PFK2 isoform PFKFB3 is highly expressed in several human cancer types, including lung adenocarcinoma, prostate cancer, and bladder cancer. The oncogenic alteration R316H in PFKFB3 can alter the regulation of its kinase activity by tyrosine kinase inhibitors. PGAM1 balances intracellular 3-phosphoglycerate and 2-phosphoglycerate levels, which regulate oxidative pentose phosphate pathway and serine synthetic pathway activities, respectively. Phosphoenolpyruvate carboxykinase (PEPCK) is highly expressed in several human cancer types, including renal cell carcinoma and hepatocellular carcinoma. The PEPCK promoter contains a CACGTC motif, which may be a target for therapeutic intervention. Fatty acids are converted to glucose or used for biomass generation. Glutamine can be used for biomass generation or for the synthesis of glutamine. Glucose is converted to acetyl-CoA, which is then used for lipid synthesis or for the synthesis of glycogen.
Organisms gauge environmental conditions to decide cell fate.
Organisms gauge environmental conditions to decide cell fate.

Nutrient sensing regulates growth in unicellular organisms.
Growth factor signals induce proliferation in multicellular organisms.
Organisms gauge environmental conditions to decide cell fate

**Unicellular organisms**
- Abundant nutrients → Proliferative metabolism
  - Sugar -> ATP + CO₂
  - Biomass + Ethanol/organic acids
- Scarce nutrients → Starvation metabolism

**Multicellular organisms**
- Abundant nutrients → Growth signal
  - Glucose -> ATP + CO₂
  - Biomass + lactate
- No growth signal → Quiescent (differentiated) cell metabolism
  - Sugar

Nutrient sensing regulates growth in unicellular organisms
Growth factor signals induce proliferation in multicellular organisms
Multicellular organisms integrate hormonal signaling

**a Unicellular eukaryote**
- Nutrient transporter
- Growth

**b Metazoan cell**
- Growth factor
- Growth

Abundant nutrients ➞ Proliferation ➞ Growth arrest
Scarce nutrients ➞ Growth arrest
Presence of signals ➞ Proliferation
Absence of signals ➞ Growth arrest
Cytokine stimulation of aerobic glycolysis in hematopoietic cells exceeds proliferative demand

Daniel E. Bauer,* Marian H. Harris,* David R. Plas, Julian J. Lum, Peter S. Hammerman, Jeffrey C. Rathmell, James L. Riley, and Craig B. Thompson

Fig. 1. IL-3 stimulation results in increases in cell number, cell size, and glycolytic rate.

A

Fl5.12 are immortalized but non-tumorigenic lymphoblastoid cells that depend on the presence of IL-3 for growth and proliferation.
Cytokine stimulation of aerobic glycolysis in hematopoietic cells exceeds proliferative demand

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Pyruvate kinase M2 is a phosphotyrosine-binding protein

Heather R. Christofk1, Matthew G. Vander Heiden1,3, Ning Wu1, John M. Asara2,4 & Lewis C. Cantley1,4

Proteomics identified that a glycolytic protein is phosphorylated in response to growth stimuli.

This protein turned out to be the M2 isoform of pyruvate kinase

Pyruvate kinase M2 is a phosphotyrosine-binding protein

Heather R. Christofk¹, Matthew G. Vander Heiden

Pyruvate kinase M2 is a glycolytic enzyme that is activated by fructose bisphosphate (FBP). It is known that a glycolytic protein is induced in response to growth stimuli. It has been found that FBP can stimulate pyruvate kinase activity and lead to glucose metabolism. The mechanism by which this is accomplished is not fully understood but it is initiated by phosphorylation of signalling proteins.

More recent studies have shown that growth factor signalling pathways can lead to changes in cell metabolism. The biochemical mechanism by which this is accomplished is not fully understood but it is initiated by phosphorylation of signalling proteins.

Using a novel proteomic screen for phosphotyrosine-binding proteins, we have made the observation that an enzyme involved in glycolysis, the human M2 (fetal) isoform of pyruvate kinase (PKM2), binds directly and specifically to phosphotyrosine. Using a novel proteomic screen for phosphotyrosine-binding proteins, we have made the observation that an enzyme involved in glycolysis, the human M2 (fetal) isoform of pyruvate kinase (PKM2), binds directly and specifically to phosphotyrosine.

To confirm the preferential binding of pyruvate kinase to phosphotyrosine, point mutants of various residues in and around the PKM2 active site were made and tested for binding to phosphotyrosine peptides. As shown in Fig. 1d, immunoblotting for both p85 and PKM2 identified a protein complex that selectively binds to phosphotyrosine peptides.

Furthermore, the binding of PKM2 to phosphotyrosine can be competed by FBP, indicating that this binding is specific to PKM2. In addition, the binding of PKM2 to phosphotyrosine can be competed by FBP, indicating that this binding is specific to PKM2.

To determine whether the FBP-binding pocket on PKM2 coordinates phosphotyrosine binding, we assessed whether FBP could compete for binding of PKM2 to a phosphotyrosine peptide. As shown in Fig. 2a, the FBP-binding pocket on PKM2 is unique to PKM2 that allows for binding of its activator, FBP.

As validation of the phosphotyrosine-binding property of pyruvate kinase, we assessed whether FBP could compete for binding of PKM2 to a phosphotyrosine peptide. As shown in Fig. 2a, the FBP-binding pocket on PKM2 is unique to PKM2 that allows for binding of its activator, FBP.

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In summary, we have identified that a glycolytic protein is induced in response to growth stimuli. We have found that this protein is the PKM2 isoform of pyruvate kinase. We have further shown that this isoform binds specifically to phosphotyrosine and is competed by FBP, indicating that this binding is specific to PKM2. This binding is likely to be involved in the regulation of glucose metabolism by growth factors.
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---

*Christofk et al, Nature, 2008*
Fast proliferating cells express an alternative form of Pyruvate Kinase
Fast proliferating cells express an alternative form of Pyruvate Kinase
Fast proliferating cells express an alternative form of Pyruvate Kinase
Fast proliferating cells express an alternative form of Pyruvate Kinase

1. Allosteric/metabolic
2. Transcription (Growth Factor Signaling)
3. Phosphorylation
PKM2 has LOWER activity
Lower PKM activity leads to accumulation of glycolytic intermediates
Glycolytic flux is regulated and provides substrates for biosynthetic pathways.
Glycolytic flux is regulated and provides substrates for biosynthetic pathways.
Estradiol stimulates the biosynthetic pathways of breast cancer cells: Detection by metabolic flux analysis

Neil S. Forbes*, Adam L. Meadows, Douglas S. Clark, Harvey W. Blanch

NMR measurements of major metabolites inside and outside (medium) the cell
Both transcriptional and post-translational changes contribute to metabolic remodeling
The hexosamine biosynthetic pathway couples growth factor-induced glutamine uptake to glucose metabolism

Kathryn E. Wellen,1 Chao Lu,1 Anthony Mancuso,1 Johanna M.S. Lemons,2 Michael Ryczko,3 James W. Dennis,3 Joshua D. Rabinowitz,4 Hilary A. Coller,5 and Craig B. Thompson1,6

FL5.12 cells supplemented with IL-3 enhance their glutamine uptake
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Glucose deprivation impairs glycosylation of IL-3R

Wellen et al., Genes Dev, 2010
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FL5.12 cells supplemented with IL-3 enhance their glutamine uptake

HexP intermediates rescue cell growth in glucose-deprived conditions

Glucose deprivation impairs glycosylation of IL-3R

Wollen et al, Genes Dev, 2010
Multi-level regulation of intracellular metabolism

Nutrient availability

Cell membrane
Multi-level regulation of intracellular metabolism

Nutrient availability

Nutrient uptake

Cell membrane
Multi-level regulation of intracellular metabolism

Nutrient availability

Nutrient uptake

Cell membrane
Multi-level regulation of intracellular metabolism

Nutrient availability

Nutrient uptake

Cell membrane

Nutrient channeling (wiring)

enz 1

enz x

enz 2
Multi-level regulation of intracellular metabolism

Nutrient availability

Nutrient uptake

Nutrient channeling (wiring)
Glucose uptake: the case of INSULIN
Insulin triggers membrane-associated GTPases and phosphatidylinositol 3-kinase
Insulin triggers membrane-associated GTPases and phosphatidylinositol 3-kinase

At rest state, more than 50% of GLUT4 is sequestered in specialized immobile storage vesicles. Signaling mobilizes these vesicles through multiple mechanisms.
Insulin triggers membrane-associated GTPases and phosphatidylinositol 3-kinase

At rest state, more than 50% of GLUT4 is sequestered in specialized immobile storage vesicles. Signaling mobilizes these vesicles through multiple mechanisms.

**GLUT4 on the PM**
++ glucose uptake
MYC mediates GLUTAMINE uptake through transcriptional upregulation of SLC1A5
Wise, DeBerardinis et al, PNAS, 2008
Figure A: Relative mRNA abundance (arbitrary units) for ASCT2, GLS1, and LDH-A under EtOH and 4-OHT conditions.

Figure B: Glutamine Uptake, showing relative uptake in arbitrary units.

Figure C: Glutaminase Flux, showing relative flux in arbitrary units.

Figure D: Glutamine-derived Lactate, showing the relationship between 2,3-[13C]lactate and [3-13C]lactate.

Figure E: Glutamine Consumed, showing the consumption over time (h) in umol/10^9 cells for EtOH and 4-OHT conditions.

Wise, DeBerardinis et al, PNAS, 2008
Vitamin B₅ supports MYC oncogenic metabolism and tumor progression in breast cancer

While MYC is recognized as a master regulator of metabolism, inducing glycolytic flux and increasing glutaminolysis among others, the true metabolic signature of these malignant subclones in the pathophysiologically relevant context of multiclonality remains unknown.

MYC<sup>high</sup> (GFP+) and MYC<sup>low</sup> (TdTomato+) clones were mixed together and injected in mice to form polyclonal tumors.

To dissect metabolites and metabolic pathways most closely associated with the individual WM clones in situ. To this end they combined desorption electro-flow focusing ionization (DEFFI)-mass spectrometric imaging (MSI) with fluorescence microscopy.
We thus utilized isotopically labeled lactate as a proxy for diminished Krebs cycle activity and lactagenic metabolism and isotopically labeled 13C-labeled isotopologues had a distribution pattern that was largely consistent with increased Krebs cycle activity in tumors. While lactate can act as a systemic carbon carrier and glutamine uptake in WM tumors shows clonal distribution (note, this is the same image as Fig. 1f, EM and NanoSIMS analysis shows heterogeneity and cycling cells and tracing [3H]glucose- or [15N]-labeled lactate. As above, we sought to investigate whether increased glucose uptake and glutamine uptake in WM tumors following [15N]glutamine boluses to mice growing the aforemen...
Uptake of Pantothenic Acid (PA, Vitamin B5) spatially correlates with MYC\textsuperscript{high} clones.

**Kreuzaler et al, Nature Metab, 2023**

**Figure a:** Fluorescence overlay shows the distribution of PA in human primary tumors. The colors represent the normalized ion intensity of PA in different tumor regions: WM\textsuperscript{mix}, WM\textsuperscript{low}, and WM\textsuperscript{high}. The scale bar indicates 5 mm.

**Figure b:** Graph showing the normalized ion intensity of PA in WM\textsuperscript{low}, WM\textsuperscript{high}, and WM\textsuperscript{mix} tumors. The box plots display statistical significance (\(*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001\)).

**Figure c:** Graph showing the normalized ion intensity of Coenzyme A in WM\textsuperscript{low}, WM\textsuperscript{high}, and WM\textsuperscript{mix} tumors.

**Figure d:** Micrographs and immunofluorescence microscopy of human primary tumors. HCl002 and STG143 are shown with overlays of PA and MYC (purple) with hemat. (blue). The scale bars indicate 2.5 mm. The color bars represent the normalized ion intensity of PA ranging from 0 to 1.
MYC^{high} (GFP+) and MYC^{low} (TdTomato+) clones were mixed together and injected in mice to form polyclonal tumors.

Isotopically-labelled pantothenate was injected in tumor bearing mice and consecutive tissue slides were images with different methods (fluorescence, electron microscopy, mass-spectrometry).
MYC$^\text{high}$ (GFP+) and MYC$^\text{low}$ (TdTomato+) clones were mixed together and injected in mice to form polyclonal tumors.

Tumor bearing mice were provided with regular chow (food) or a diet deficient of PA.
Multi-level regulation of intracellular metabolism

Nutrient availability

Nutrient uptake

Cell membrane
Multi-level regulation of intracellular metabolism

Nutrient availability

Nutrient uptake

Cell membrane
Multi-level regulation of intracellular metabolism

Nutrient availability

Nutrient uptake

Nutrient channeling (wiring)
Multi-level regulation of intracellular metabolism

Nutrient availability

Nutrient uptake

Cell membrane

Nutrient channeling (wiring)
Glucose and glutamine carbons can be differentially utilized for anabolic purposes
PI3K/AKT signal transduction coordinates reprogramming of cell metabolism
PI3K/AKT signal transduction coordinates reprogramming of cell metabolism
PI3K/AKT signal transduction promotes glucose metabolism (glycolysis)

AKT activates Hexokinase that activates GLC, locking sugars for catabolism
PI3K/AKT signal transduction enhances nucleotide synthesis
PI3K/AKT signal transduction enhances FA synthesis

AKT / Protein Kinase B (PKB) is a Sereine/Threonine Kinase activated by many TM receptor through phosphatidylinositol-3-kinase (PI3K). AKT is hyper activated in about 80% of human cancers (also in Proteus syndrome, aka “elephant man”).

AKT actions:
• Activates glucose uptake and glycolysis
• Activates ACLY
• Activates mTORC1 (cell anabolism)
• Promotes cell growth and survival
• Promotes cancer

**Fig. 3** Glucose uptake, AKT actions and PI3K/AKT

- Activates glucose uptake and AKT actions
- AKT is hyper activated in about 80% of human cancers (also in Proteus syndrome, aka “elephant man”).

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**Fig. 3** Glucose uptake, AKT actions and PI3K/AKT

- Activates glucose uptake and AKT actions
- AKT is hyper activated in about 80% of human cancers (also in Proteus syndrome, aka “elephant man”).
Akt-Dependent Metabolic Reprogramming Regulates Tumor Cell Histone Acetylation

Joyce V. Lee,1,2,11 Alessandro Carrer,1,2,11 Supriya Shah,1,2,11 Nathaniel W. Snyder,3 Shuanzeng Wei,4 Sriram Venneti,5 Andrew J. Worth,6 Zuo-Fei Yuan,6 Hee-Woong Lim,7 Shichong Liu,8 Ellen Jackson,1,2 Nicole M. Aiello,2,8 Naomi B. Haas,8 Timothy R. Rebbeck,9 Alexander Judkins,10 Kyong-Jae Won,7 Lewis A. Chodosh,1,8 Benjamin A. Garcia,6 Ben Z. Stanger,2,8 Michael D. Feldman,4 Ian A. Blair,3 and Kathryn E. Wellen1,2,*

INTRODUCTION

Increased macromolecular biosynthesis, growth, and proliferation by oncogenic metabolic reprogramming. Here, we show that metabolic enzymes may also promote tumorigenesis.

[Graphs and tables showing results of experiments related to Akt activation and its effects on histone acetylation and metabolic enzymes.]
Cells with constitutively active AKT (myr-AKT) have sustained ACLY phosphorylation and more abundant lipid species

Lee, Carrer et al, *Cell Metab*, 2014

Porstmann et al, *Oncogene*, 2005
Hypoxia-inducible factors (HIFs) are transcription factors that control the cell response to hypoxia.

In presence of oxygen, prolyl hydroxylases (PHDs) target HIF for degradation (*interestingly aKG, Fe2+ and ascorbate are cofactors for this reaction*).

When oxygen becomes limited, HIFs are no longer degraded and can act.

HIF actions:
- Activate glucose uptake and glycolysis
- Inhibit pyruvate entry into mitochondria
- Balance intracellular pH (drops in hypoxia)
- Promote erythropoiesis
- Promote angiogenesis

Hypoxia induces switch to glycolytic metabolism.
Kim et al., Cell Metabol, 2006

A

PDK1
Actin
Time (h) 22 29 48
Hypoxia
22 29 48
Normoxia

B

PDK1
loading
Time (h) 22 29 48
CoCl$_2$
22 29 48
Control

C

PDK1
HK2
Actin
Time (h) 24 48 72
WT MEF
24 48 72
Hif1a/- MEF
24 48 72
WT MEF
24 48 72
Hif1a/- MEF

D

% of total input

Black: Anti-HIF1α antibody
White: Control IgG

Hypoxia
Normoxia

consensus HIF1 binding site

amplicons

1 2 3 4

-2kb +1 +2kb

Kim et al., Cell Metabol, 2006
CONCLUSIONS (1)

Growth and proliferation need nutrients AND signals
Nutrient uptake and usage are REGULATED by signal transduction
Most growth signals induce nutrient uptake and anabolic pathways
Nutrient uptake is DIVERSE
AKT and MYC promote similar yet distinct metabolic reprogramming
In response to hormones and growth factors, PI3K signaling network functions as a major regulator of metabolism and growth, governing (...). Many of the driver mutations in cancer with the highest recurrence, including (...), pathologically activate PI3K signaling. However, our understanding of the core metabolic program controlled by PI3K is almost certainly incomplete.
PI3K drives the de novo synthesis of coenzyme A from vitamin B5

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PI3K drives the de novo synthesis of coenzyme A from vitamin B5

**Diagram:**

```
VB5
| ATP | ADP | PANK1-3 |
| 4'-Phosphopantothenate |
| Cysteine | ATP | AMP + PP | PPCS |
| 4'-Phosphopantothenoylcysteine |
| H⁺ | CO₂ | PPCDC |
| 4'-Phosphopantetheine |
| ATP | PPi | 3'-Dephospho-CoA |
| ATP | ADP | CoA |
```

**Table:**

<table>
<thead>
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<th>Enzyme</th>
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<th>Substrate quality</th>
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<td>R L R R R M D S G R</td>
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<tr>
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<td>S169</td>
<td>P L R R R A S S A S</td>
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<td>V S R Q R V E S L R</td>
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<tr>
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<td>PANK4</td>
<td>T406</td>
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<tr>
<td>AKT substrate motif:</td>
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</table>

**Caption:**

In vitro treatment: Cells were treated with insulin to acutely stimulate PI3K signalling. We stably reconstituted cells and derivative lines stably reconstituted with PANK2, we were able to study the effects further. We generated data and Fig. 2 | PANK2 and PANK4 are direct AKT substrates. Amino acid sequence was the major source of CoA (Extended Data Fig. 4). CoA synthesis is mostly attributed to the CoA synthesis pathway that are enzymes of the CoA synthesis pathway that are responsible for CoA synthesis (Fig. 3). p-PRAS40 (T246) is uniquely used in combination with cysteine and ATP for dephosphorylation. Cells were treated with insulin to acutely stimulate PI3K signalling. MCF10A cells are similarly dependent on CoA abundance, but the molecular regulatory mechanisms underlying this context (Extended Data Fig. 5a) to individually deplete PANK2 and PANK4 using the non-transformed human breast epithelial cell line MCF10A. PI3K has important roles in mammary gland growth and is a key target for cancer treatment. Cells were treated with insulin to acutely stimulate PI3K signalling. MCF10A cells are similarly dependent on CoA abundance, but the molecular regulatory mechanisms underlying this context (Extended Data Fig. 5a) to individually deplete PANK2 and PANK4 using the non-transformed human breast epithelial cell line MCF10A. PI3K has important roles in mammary gland growth and is a key target for cancer treatment.
PI3K drives the de novo synthesis of coenzyme A from vitamin B5

**Diagram:**
- Knockdowns (validated in Extended Data Fig. 4)

**Figure 4:**
- Knockdowns (validated in Extended Data Fig. 4)
- C57BL/6J treated with vehicle or PI3K inhibitor (BYL-719, 50 µM) treatments (30 minutes)
- Mouse allograft mammary tumour treatment:
  - C57BL/6J treated with vehicle or PI3K inhibitor (BYL-719, 50 µM) treatments (30 minutes)
- Mouse allograft mammary tumour treatment:
  - C57BL/6J treated with vehicle or PI3K inhibitor (BYL-719, 50 µM) treatments (30 minutes)
- Mouse allograft mammary tumour treatment:
  - C57BL/6J treated with vehicle or PI3K inhibitor (BYL-719, 50 µM) treatments (30 minutes)

**Table:**
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PI3K drives the de novo synthesis of coenzyme A from vitamin B5

**Article**

**b**

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<td>AKT substrate motif:</td>
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**c**

**IP Ab:** IgG | PANK

**Insulin:**

- | + | + | +

**GDC-0941:**

- | - | - | +

**PANK1 IP**

- **p-RXRXXS/T**
  - **PANK1**
  - **PANK2**
  - **PANK4**

**PANK2 IP**

- **p-RXRXXS/T**
  - **PANK2**
  - **PANK4**

**PANK4 IP**

- **p-RXRXXS/T**
  - **PANK4**

**Lysate**

- **p-AKT (T308)**
  - **AKT**
  - **PANK1**
  - **PANK2**
  - **PANK4**

**i**

- **p-S169**
  - **p-S189**

- **PANK2 N**
  - **Pantothenate kinase**
  - **p-T406**

- **PANK4 N**
  - **Pseudokinase**
  - **DUF89 phosphatase**

**Dibble et al, Nature, 2022**
PI3K drives the de novo synthesis of coenzyme A from vitamin B5

Growth factors
Hormones

PI3K

AKT

PANK2

PANK4

ACLY

Glucose

Glycolysis

Citrate

CoA

Acetyl-CoA

VB5

Uptake

?-P

?-P
New layers of metabolic regulation are being constantly discovered. Different signaling pathways converge to the same metabolic goal.
Inborn errors of metabolism (IEM)

- Genetic (loss of function)
- Almost all are autosomal or X-linked recessive
- Large class of congenital disorders. Individually rare, but collectively affect ca. 1:1500
- Multi-organ system dysfunction – organs with prominent roles in metabolic regulation (e.g. liver) or high metabolic demand (brain, muscle) are often involved.
- Progressive
- Some are treatable

![Diagram of normal and Pompe affected cells with GAA: acid alpha-glucosidase]
Inborn errors of metabolism (IEM)

- Genetic (loss of function)
- Almost all are autosomal or X-linked recessive
- Large class of congenital disorders. Individually rare, but collectively affect ca. 1:1500
- Multi-organ system dysfunction – organs with prominent roles in metabolic regulation (e.g. liver) or high metabolic demand (brain, muscle) are often involved.
- Progressive
- Some are treatable
Multi-level regulation of intracellular metabolism

Nutrient availability

Nutrient uptake

Nutrient channeling (wiring)
Tissue metabolism dictates nutrient availability

1. Dietary intake dictates local abundance of metabolites in peripheral tissues

SYSTEMIC/TISSUE RELATIONSHIP
Tissue metabolism dictates nutrient availability

**SYSTEMIC/TISSUE RELATIONSHIP**

1. Dietary intake dictates local abundance of metabolites in peripheral tissues

**METABOLIC COMPETITION**

2. Different cell types often compete for the same nutrients.

- T cell
- MDSC
- Tumor

- ATP loss
- IDO upregulation in DCs

- Glucose → Amino acids → Fatty acids

- T cell → Treg → MDSC → Tumor

- PD-L1 → ↑ Metabolism
Tissue metabolism dictates nutrient availability

1. Dietary intake dictates local abundance of metabolites in peripheral tissues

2. Different cell types often compete for the same nutrients.

3. Nutrients can be provided by a different cell type in the tissue

- Systemic/Tissue Relationship
- Metabolic Competition
- Metabolic Symbiosis
Metabolic Interactions

COOPERATIVE

Blood vessel
- glucose → lactate

Astrocyte
- glutamate

Neurons
- glutamate

COMPETITIVE

Peripheral Tissue
- Tryptophan

Effector T cells
Metabolic Interactions

**COOPERATIVE**
- Blood vessel
  - glucose → lactate
  - angiogenesis
- Astrocyte
- Neurons
  - glucose
  - glutamate
  - polarization
- Macrophage

**COMPETITIVE**
- Tryptophan
  - Effector T cells
  - Peripheral Tissue
Metabolic Interactions

COOPERATIVE

Blood vessel
- glucose → lactate
- angiogenesis

Astrocyte
- glutamate

Neurons

Macrophage
- polarization

COMPETITIVE

Tryptophan
- Kynurenine

Peripheral Tissue

Effector T cells
- Regulatory T cells
Obesity Shapes Metabolism in the Tumor Microenvironment to Suppress Anti-Tumor Immunity
Local nutrient availability is dictated cell-cell interplay
Nutrient competition is a physiological feedback mechanism
Nutrient cooperation is a physiological mechanism of adaptation
Local nutrient availability is different across different tissues and changes according to multiple systemic inputs
Proliferation presents metabolic challenges

**Diagram:**
- **Quiescent cell**
  - Glucose → Glycolysis → ATP → TCA cycle → CO₂ → Glutamine → Glutaminolysis
- **Proliferating cell**
  - Glucose → Glycolysis → ATP → TCA cycle → CO₂ → Glutamine → Glutaminolysis
  - Fatty acids
  - To biomass

The diagram illustrates the metabolic pathways in quiescent and proliferating cells. In quiescent cells, glucose is metabolized through glycolysis, producing ATP, TCA cycle, CO₂, and glutamine via glutaminolysis. In proliferating cells, the process is similar but with an increased flow to biomass, indicating higher energy needs for cell proliferation.

**References:**

Finley LY, Thompson CB, *Cell*, 2012
Proliferation presents metabolic challenges

**Diagram Description**

- **Quiescent cell**
  - Glucose enters the cell and undergoes glycolysis to produce ATP and CO₂.
  - Fatty acids are used for energy production.
  - Glutamine is used for glutaminolysis.
  - TCA cycle (Tricarboxylic Acid Cycle) processes intermediates.

- **Proliferating cell**
  - Glucose is the primary energy source and enters the cell through glucose transporters.
  - Fatty acids and glutamine are used as energy sources.
  - Glycolysis generates ATP and pyruvate, which can be oxidized to produce more ATP.
  - The TCA cycle and oxidative phosphorylation are accelerated to support rapid cell division.
  - Proliferating cells have higher rates of protein synthesis and amino acid uptake.

**Key Enzymes and Pathways**

- **Pyruvate dehydrogenase (PDH)**
- **Malate dehydrogenase (MDH)**
- **Isocitrate dehydrogenase (IDH)**
- **Fumarate hydratase (FH)**
- **Succinate dehydrogenase (SDH)**

**Metabolic Pathways**

- **GLYCOCYSIS**
  - Conversion of glucose to pyruvate.
  - Production of ATP through glycolysis.
- **GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PDH)**
- **6-PHOSPHOGLUCONATE DEHYDROGENASE (6PGDH)**
- **PHOSPHOGLUCONATE PHOSPHATASE (PGAM1)**

**Regulatory Factors**

- **Akt**
- **PI3K**
- **PKM2**
- **FRM2**
- **PHGDH**

**Pathway Enhancements**

- **PGAM1** balances intracellular 3-phosphoglycerate and 2-phosphoglycerate levels, which regulate oxidative pentose cycle.

**Clinical Relevance**

- FH mutations lead to increased risk for certain cancers.
- IDH mutations are associated with better survival outcomes in some cancers.
- Cancer cells exhibit altered metabolic pathways compared to normal cells.

**References**

Finley LY, Thompson CB, *Cell*, 2012
Proliferating cells have increased demand for glycolytic intermediates

Differentiated tissue

- Glucose $\rightarrow$ Pyruvate $\rightarrow$ Lactate $\rightarrow$ CO$_2$
  - Oxidative phosphorylation
    - $-36$ mol ATP/mol glucose

Proliferative tissue

- Glucose $\rightarrow$ Pyruvate $\rightarrow$ Lactate
  - Anaerobic glycolysis
    - 2 mol ATP/mol glucose
  - or
    - Aerobic glycolysis (Warburg effect)
      - $-4$ mol ATP/mol glucose

Tumor

- Glucose $\rightarrow$ Pyruvate $\rightarrow$ Lactate $\rightarrow$ CO$_2$

Proliferating cells have increased demand for glycolytic intermediates
Proliferating cells need nucleotides

Table 1. (continued)

<table>
<thead>
<tr>
<th>Regulators/signaling proteins/nutrients</th>
<th>Mechanism(s)</th>
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<td>Energy stress regulates purine synthesis, possibly through AMPK-dependent sequestration of PFAS from purinosome metabolon</td>
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<td>Urea cycle and CAD</td>
<td>In cancers with decreased ASS1, reduced usage of aspartate by urea cycle leads to accumulation of cytosolic aspartate pools that can be consumed by CAD to boost pyrimidine synthesis</td>
<td>[65]</td>
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Abbreviations: AMPK, AMP-activated protein kinase; ARL13B, ADP-ribosylation factor-like protein 13B; ASS1, argininosuccinate synthase; BTIC, brain tumor-initiating cells; CPS1, carbamoyl phosphate synthetase-1; GLUL, glutaminase; IMPDH, inosine monophosphate dehydrogenase; MTHFD2, methylene tetrahydrofolate dehydrogenase 2; PTEN, phosphatase and tensin homolog; RPIA, ribose 5-phosphate isomerase A; RRM2, ribonucleotide reductase member 2; SIRT3, NAD-dependent deacetylase sirtuin 3, mitochondrial; SLC4A7, solute carrier family 4 member 7; SREBP1, sterol regulatory element-binding transcription factor 1.

Figure 2. Regulation of de novo purine and pyrimidine synthesis by the cell signaling networks.

(A) In response to growth factors, mechanistic target of rapamycin complex I (mTORC1) is activated downstream of the phosphatidylinositol 3-kinase (PI3K)/Akt pathway and promotes S6K1-dependent phosphorylation of carbamoyl-phosphate synthetase 2 (CPS2), aspartate transcarbamoylase (ATC), and dihydroorotase (DHO) (CAD), thereby increasing de novo pyrimidine synthesis. Moreover, mTORC1 activation fuels de novo purine and pyrimidine synthesis by increasing cellular bicarbonate abundance through the stimulation of solute carrier family 4 member 7 (SLC4A7) mRNA translation via the S6K-dependent phosphorylation of eukaryotic translation initiation factor 4B (eIF4B). Downstream of mTORC1, the transcription factor sterol regulatory element-binding protein 1 (SREBP1) stimulates the oxidative pentose phosphate pathway (PPP) to enhance de novo purine and pyrimidine synthesis via increased availability of 5′-phosphoribosyl-pyrophosphate (PRPP). Additionally, mTORC1 activation enhances activating transcription factor 4 (ATF4) expression, promoting serine/glycine synthesis and N-formyl-THF production, which increases de novo purine synthesis. (B) In response to growth factors, extracellular signal-regulated kinase (ERK) phosphorylates CAD and phosphoribosylformylglycinamidine synthase (PFAS), enhancing de novo pyrimidine or purine synthesis, respectively. MYC, a downstream transcription factor of RAS-ERK, controls the expression of genes involved in de novo purine and pyrimidine synthesis. (C) Insulin activates the PI3K-Akt pathway, which leads to mTORC1 activation. In addition, Akt activates the nonoxidative PPP through transketolase (TKT) phosphorylation, boosting PRPP synthesis for nucleotide synthesis. Abbreviations: EGF, epidermal growth factor; IGF1, insulin-like growth factor 1; RPIA, ribose 5-phosphate isomerase A.
Proliferating cells have increased demand for glycolytic intermediates

Post-mitotic differentiated cells focus on efficient oxidative metabolism to extract the maximum amount of ATP from nutrients -> ‘manning the pumps’ (ion channels) and executing specialized functions

Proliferating cells (development, immune system, cancer) rewire metabolism to support the biomass accumulation required for cell division; this funnels intermediates through all of the biosynthetic hubs we discussed earlier
Proliferating cells have increased demand for reducing equivalents
(support anapldrotic reactions and regeneration antioxidants)

Adapted from: Vander Heiden M. Nat Rev Drug Disc 2011
Proliferating cells have increased demand for reducing equivalents
(support anapldrotic reactions and regeneration antioxidants)

Non-proliferating Cells

- Glucose
- Glutamine
- ATP
- NADPH

Proliferating Cells

- Glucose
- Glutamine
- Lipids
- Carbohydrates
- Protein
- Nucleic acid
- NADPH

Chemotherapy “Anti-metabolites”

…rate limiting for cellular proliferation

Adapted from: Vander Heiden M. Nat Rev Drug Disc 2011
The cofactor NADPH provides high-energy electrons for antioxidant defense and reductive biosynthesis.

Consumption and production of NADPH is compartmentalized, with cytosolic NADPH used by enzymes including fatty acid synthase, ribonucleotide reductase, thioredoxin reductase, and glutathione reductase.

Regeneration of cytosolic NADPH from NADP occurs by three well-validated routes (each ubiquitously expressed in mammals):
- malic enzyme 1 (ME1),
- isocitrate dehydrogenase 1 (IDH1),
- the oxidative pentose phosphate pathway (oxPPP).
Figure 1. G6PD is required to maintain cell growth, NADPH/NADP ratio, and redox defense.

(a) Schematic of cytosolic NADPH production pathways. (b) Western blot for G6PD, IDH1, and ME1 protein levels in clonal HCT116 deletion cell lines. (c) Normoxic and (d) hypoxic growth (0.5% O$_2$) of clonal HCT116 deletion cell lines lacking the indicated NADPH production enzymes in DMEM (plated at 2500 cells per well, n = 4). Note that ΔG6PD/ΔME1 cells die at low cell density, (e) NADPH and (f) NADP concentration. (g) NADPH/NADP ratio and (h) relative GSH/GSSG ratio (n = 3 for e-h). (i,j) Relative live cells after H$_2$O$_2$ or diamide treatment for 3 days, normalized to untreated cells (n = 6). Data are mean ± SD with n indicating the number of biological replicates. *p < 0.05 and **p < 0.01 by one-way ANOVA with Dunnett’s multiple comparison correction (see Supplementary Table 3 for exact P values).

Figure 4. Fatty acid synthesis is maintained in HCT116 G6PD knockout cells.

(a) Fraction of different non-essential fatty acid species synthesized de novo based on extent of labeling from U-$^{13}$C-glucose (for full labeling patterns, see Supplementary Fig. 3, n = 2). (b) NADPH consumption for fatty acid synthesis (n = 3). (c,d) Palmitoleic acid (C16:1) and citrate labeling fraction in cells fed 100 µM [U-$^{13}$C]palmitate (conjugated to BSA) for 24 h (n = 6). (e) NADPH production by the oxPPP (measured using $^{14}$C-CO$_2$ release from [1-$^{14}$C] and [6-$^{14}$C]glucose, n = 4) and the upper limit of production by ME1 (measured based on [U-$^{13}$C]-lactate production from [U-$^{13}$C]glutamine, n = 3) and NADPH consumption flux for fatty acid synthesis (n = 3). Data are mean ± SD with n indicating the number of biological replicates.

Figure 6. Across cell lines, G6PD knockout consistently causes folate deficiency.

(a) Heat map showing intracellular levels of water-soluble metabolites in G6PD deletion cells. For each cell line, three or four individual biological replicates are shown, normalized to respective WT cells. (b) Relative levels of NADPH, NADP, malate, (iso)citrate, dUMP (n = 3 for HCT116 and HEK293T, n = 4 for others), and DHF (n = 3). Note that the dUMP and DHF panels are on a logarithmic Y-axis. (c) Schematic: G6PD deletion leads to accumulation of NADP, DHF and dUMP. Data are mean ± SD with n indicating the number of biological replicates, ns p $\geq$ 0.05, * p < 0.05 and ** p < 0.01 by two-tailed paired t-test assessing whether G6PD KO cell lines (as a group) differs from WT, with the geometric mean value for each cell type treated as one sample and n = 6 the number of cell lines (see Supplementary Table 3 for full statistical parameters.)
Figure 1. G6PD is required to maintain cell growth, NADPH/NADP ratio, and redox defense.

Figure 2. Fatty acid synthesis is maintained in HCT116 G6PD knockout cells.

Figure 3. Across cell lines, G6PD knockout consistently causes folate deficiency.

Figure 4. Fatty acid synthesis is maintained in HCT116 G6PD knockout cells.

Figure 5. NADPH production by the oxPPP (measured using [14C] and [6-13C] lactate production from [U-13C]glucose).

Figure 6. Across cell lines, G6PD knockout consistently causes folate deficiency.
Figure 1. G6PD is required to maintain cell growth, NADPH/NADP ratio, and redox defense.

(a) Western blot for G6PD, IDH1, ME1, and ME1 protein levels in clonal HCT116 deletion cell lines. (b) Relative NADPH/NADP ratio and (Δ) NADP concentration. (c) Relative GSH/GSSG ratio.

(b) Schematic of cytosolic NADPH production pathways. (Δ) Production enzymes in DMEM (plated at 2500 cells per well, n = 4). Note that ME1 cells die at low cell density, (Δ) Relative live cells after 3 days of normoxia or diamide treatment, normalized to untreated cells (n = 6). Data are mean ± SD with n indicating the number of biological replicates. *p < 0.05 and **p < 0.01 by one-way ANOVA with Dunnett's multiple comparison correction (see Supplementary Table 3 for full statistical parameters).
Across cell lines, G6PD knockout consistently causes folate deficiency. (Supplementary Table 3 for full statistical parameters.)

Data are mean ± SD with n indicating the number of biological replicates. *p < 0.05 and **p < 0.01 by one-tailed paired t-test, based on [1-14C]glucose, n = 4 and [6-14C]glucose (for full labeling patterns, see Supplementary Fig. 3, n = 2).
NADPH is Devoted to Support Growth...

...Opening Vulnerabilities in Redox Targeting

Energy Metabolism in Normal Cells

Glycolysis → NADH → Respiration

Glu → Aspartate → aKG → MDH1 → NADH → OGC1 → Malate → aKG

NADH → Glu → OAA → GOT1 → NAD+ → MDH2 → NADH

Glu → OAA → GOT2 → NAD+ → MDH1 → NADH

Cytosol | Mitochondria

Oncogene (Kras)-mediated Rewiring of Pancreatic Cancer Metabolism

Glycolysis → NADH → NAD⁺ → Respiration

Glu → Aspartate → OAA → Malate → OGC1 → Malate → aKG → NAD⁺ → NADPH

Gln → Glu → TCA cycle → Glu → Aspartate → AGC1

Oncogene (Kras)-mediated Rewiring of Pancreatic Cancer Metabolism

Proliferating cells enable anaplerosis from non-canonical carbon sources

Quiescent cells

Proliferating cells

Glucose → TCA cycle → Lactate

Glucose → Lipids → Proteins

Glutamine → TCA cycle → Lactate
Proliferating cells enable anaplerosis from non-canonical carbon sources

**Quiescent cells**

- Glucose
- Lactate
- TCA cycle
- Glutamine

**Proliferating cells**

- Glucose
- Lactate
- TCA cycle
- Glutamine
- Lipids
- Proteins
**ANAPLEROSIS vs CATAPLEROSIS**

**Anaplerosis** is a series of enzymatic reactions in which metabolic intermediates enter the TCA cycle from the cytosol.

**Cataplerosis** is the opposite. A process where intermediates leave the TCA cycle (and mitochondria).

It implies if a C atom (CO2) replenishes or not the TCA cycle.
Mitochondria couple pyruvate oxidation, electron transport and oxidative phosphorylation.
The TCA cycle at the crossroad of catabolism and anabolism.
Aspartate is a precursor for nucleotide synthesis and is indispensable for cell proliferation. Moreover, the malate–aspartate shuttle plays a key role in redox balance, and a deficit in aspartate can lead to oxidative stress. It is now recognized that aspartate biosynthesis is largely governed by mitochondrial metabolism, including respiration and glutaminolysis in cancer cells.
In vitro
In vivo??
Targeting mitochondrial genes impairs tumor growth

**Figure 1 | Complex III is necessary for tumour growth.**

A) Scheme of the mitochondrial electron transport chain (ETC) and complex III function in T-ALL cells (Extended Data Fig. 1a). DHODH expression in 143B-∆ cells alleviated their survival (Extended Data Fig. 1b). As a result, AOX restored the basal OCR in 143B-∆ cells (Fig. 1a). B) Tumour volume of 143B-CYTB cells (Fig. 1b). Values are means ± s.e.m. Survival curves (Fig. 1b) with a Bonferroni test for multiple comparisons (exact Poisson, survival comparisons). **P < 0.01. 

*Martinez-Reyes et al, Nature, 2020*
Mitochondria couple pyruvate oxidation, electron transport and oxidative phosphorylation
Targeting mitochondrial genes impairs tumor growth

Complex III deficiency suppresses:
- ATP synthesis
- Proton pumping
- Electron transport
- TCA cycle
- CoQ oxidation (necessary for DHODH activity in pyrimidine biosynthesis)

Mitochondrial ATP production is NOT essential for tumor growth

AOX expression (in C3-KO tumors) re-establish fully functional C1-C2 activity, only modestly rescues proton pumping, ATP synthesis

Mitochondrial ATP production is NOT essential for tumor growth

CoQ oxidation and TCA cycle are most important for tumor growth

Martinez-Reyes et al, Nature, 2020
CONCLUSIONS (3)

Proliferating cells have distinct metabolic demands
Metabolism influences proliferation
Critical: lipids and nucleotides. Biosynthesis requires NADPH.
Despite being highly glycolytic, proliferating cells need mitochondria
In Vitro and In Vivo Metabolism

Glycolysis

Glucose → Pyruvate → ATP → Lactate

Mitochondrial Gln Metabolism

Glucose → O_2 → ATP → Gln → TCA → Lactate → OXPHOS

FAO
Critical differences in metabolism observed in vitro and in vivo
Critical differences in metabolism observed \textit{in vitro} and \textit{in vivo}

AKA: metabolism is context-dependent
AKA-bis: studying metabolism is challenging
Critical differences in metabolism observed *in vitro* and *in vivo*

AKA: metabolism is context-dependent
AKA-bis: studying metabolism is challenging

Cell culture (plates)  Animals
Critical differences in metabolism observed in vitro and in vivo

AKA: metabolism is context-dependent

AKA-bis: studying metabolism is challenging

Cell culture (plates)  Animals

Figure 6. Lung Cancer Cells Require Pdha1 for Tumor Formation In Vivo

(A) sgRNAs targeting Pdha1 were introduced into lung tumor tissues. Proliferation of control and Pdha1-disrupted cells is shown. Scale bar, 200 μm.

(B) The same cells described in (A) were cultured in the presence of [U-13C5]glucose infusion. The M0 and M2 isotopomers are shown for each metabolite. All values in mean ± SEM. *Difference is statistically significant by two-tailed Student’s t test compared to control, n = 3 per condition.

(C)glucose infusion. The M0 and M2 isotopomers are shown for each metabolite. All values in mean ± SEM. *Difference is statistically significant by two-tailed Student’s t test compared to control, n = 3 per condition.

(D) The same cells described in (A) were introduced as allografts into the flanks of mice. The percent labeling of aspartate (Asp), citrate (Cit), glutamine (Glu), and oxaloacetate (OAA) in lung tumors derived from control cells (black) or material present at the injection site from Pdha1-disrupted cells (blue) was determined following glucose infusion. The percent labeling is presented in blue, and percent labeling of other metabolites is shown in orange and yellow. All values in mean ± SEM. *Difference is statistically significant by two-tailed Student’s t test compared to control, n = 3 per condition.

(E) The same cells described in (A) were introduced as allografts into the flanks of nu/nu mice. Immunohistochemistry assessing Pdha1 expression in lung tumors arising in KP mice infected with pSECC containing a control sgRNA (Control) or sgPdha1 (sgPcx).

(F) Representative H&E staining of the same cells described in (A) 4 weeks after orthotopic transplantation into the lungs of nu/nu mice. Scale bar, 20 μm.

(G) Representative immunohistochemical staining for Pdha1 in tumors arising in KP mice infected with pSECC containing a control sgRNA (Control) or sgPdha1 (sgPcx).
Cell culture media composition is extremely different from plasma

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Cell culture media composition is extremely different from plasma

Figure 1. Metabolic reactions observed in cancer cells cultured in historic and physiological media. Arrows and names highlighted in red indicate reactions or metabolite levels enhanced in historic media, such as DMEM. Nutrients and metabolites with a dashed outline are absent in DMEM. 5-FU: 5-fluorouracil, AcCoA: acetyl-Coenzym A, ASS: argininosuccinate, ATP: adenosine triphosphate, Citn: citrulline, Fum: fumarate, FUMP: 5-fluorouracil monophosphate, HIF1α: hypoxia-inducible factor 1α, αKG: α-ketogluterate, MetF: metformin, NAD: nicotinamide adenine dinucleotide, Oaa: oxaloacetate, Orn: Ornithine, Pyr: pyruvate, UMPS: uridine monophosphate synthetase.
Physiologic Medium Rewires Cellular Metabolism and Reveals Uric Acid as an Endogenous Inhibitor of UMP Synthase

Jason R. Cantor,1,2,3,4* Monther Abu-Remaileh,1,2,3,4 Naama Kanarek,1,2,3,4 Elizaveta Freinkman,1 Xin Gao,1,5 Abner Louissaint Jr.,2,3 Caroline A. Lewis,1 and David M. Sabatini1,2,3,4

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Graphical Abstract

Established synthetic cell culture media

Systematically developed human plasma-like medium (HPLM)

Cellular Metabolism

Established media Most mammals

Low uric acid RPMI

UMPS

Uric acid

High uric acid HPLM

HPLM

Higher primates

Increased S-FU activation

Decreased S-FU activation

Increased S-FU activation

Decreased S-FU activation

Cell death

Uric acid is an endogenous inhibitor of UMPS

Uric acid influences S-FU-mediated cytotoxicity

HPLM

HPLM (HPLM-URS)

HPLM (no uric acid)

Fraction viable cells

5-FU EC50 (μM)

[5-FU] (μM)

E

NOMO1

HPLM-URS (uric acid) HPLM-URS

Improving the metabolic fidelity of cancer models with a physiological cell culture medium

Johan Vande Voorde1, Tobias Ackermann1, Nadja Pfetzer1, David Sumpton1, Gillian Mackay1, Gabriela Kalna1, Colin Nixon1, Karen Blyth1,2, Eyal Gottlieb1,3, Saverio Tardito1,2,5

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Physiologic Medium Rewires Cellular Metabolism and Reveals Uric Acid as an Endogenous Inhibitor of UMP Synthase

Jason R. Cantor,1,2,3,6 Monther Abu-Ramiah,1,2,3,6 Naama Kanarek,1,2,3,6 Elizaveta Freinkman,1 Xin Gao,1,6 Abner Louissant, Jr.,6 Caroline A. Lewis,1 and David M. Sabatini1,2,3,4,6

INTRODUCTION

Cell number

Incubation DMEM-F12 Plasmax

BT549

CAL-120

MD-MB-468

E

NOMO1

HPLM-Δuracil (-uric acid) HPLM-Δuracil

Fraction viable cells

5-FU EC₅₀ (µM)

0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7

0 5 10 15 20 25

[5-FU] (µM)

0

0.1 1 10 100 1000

BT549

P = 0.032

P = 0.068

Medium

D P D P

O₂

N N H H

0

100,000

200,000

0

200,000

400,000

Improving the metabolic fidelity of cancer models with a physiological cell culture medium

Johan Vande Voorde1, Tobias Ackermann1, Nadja Pfitzer1, David Sumpton1, Gillian Mackay1, Gabriela Kalna1, Colin Nixon1, Karen Blyth1,2, Eyal Gottlieb1,3, Saverio Tardito1,2,6

Cells to grow and proliferate (A) that were not designed to reproduce the physiological cellular environment (B). (C) The metabolic wiring in human plasma is more complex than that of traditional culture media nor mouse plasma (D) and better recapitulates the composition of human therapeutic agent 5-fluorouracil. Thus, media that address the need for large amounts of medium with less end of a proliferation assay with BT549, CAL-120, and MDA-MB-468 cells, performed in Plasmax or DMEM-F12 under normoxic (N) and hypoxic (H) condition

Values refer to a two-tailed t-test with equal variance. *P < 0.05.
Physiologic Medium Rewires Cellular Metabolism and Reveals Uric Acid as an Endogenous Inhibitor of UMP Synthase

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Highlighting changes in metabolic pathways and drug responses

Improving the metabolic fidelity of cancer models with a physiological cell culture medium

Johan Vande Voorde, Tobias Ackermann, Nadja Pfeifer, David Sumpton, Gillian Mackay, Gabriela Kalna, Colin Nixon, Karen Blyth, Eyal Gottlieb, Saverio Tardito

Evaluating the effects of uric acid on cancer cell proliferation

Cantor et al., Cell, 2017

Vande Voorde et al., Sci Adv, 2019
Metabolism is DYNAMIC.
Cells need to reprogram their metabolism in order to:

- Produce more biomass (cell division; cell growth)
- Produce more nucleotides (cell division; meiosis)
- Preserve energy (storage; response to nutrient scarcity)
- Cope with (oxidative) stress (replication and nutrient stress)
- Compartmentalize toxic metabolites (iron overload)
- Adapt to different environments (mobility, 3D growth)
- Secrete immunomodulatory molecules (immune response)
- Adjust availability of “signaling metabolites” (support signals)
- Support epigenetic rewiring (differentiation)

…NOT to “produce” more energy
Cells reprogram their metabolism: anabolic and catabolic pathways are rewired to tackle different needs

**SIGNALING PATHWAYS (ONCOGENES)**

- ATP, GTP, Bioenergetics
- NAD⁺(H), NADP⁺(H), FAD(H₂), Redox balance
- Reprogrammed cancer metabolism
- Biomass: Protein, Lipids, Nucleic acids
- Differentiation
- Chromatin/epigenetics
Cells reprogram their metabolism: anabolic and catabolic pathways are rewired to tackle different needs

**SIGNALING PATHWAYS**

**(ONCOGENES)**

- Bioenergetics
- Redox balance
- Reprogrammed cancer metabolism
- Biomass
- Differentiation
- Microenvironment

- ATP
- GTP
- Bioenergetics
- Protein
  - Lipids
  - Nucleic acids
- NAD\(^+\)(H)
- NADP\(^+\)(H)
- FAD\(\text{H}_2\)

**Microenvironment**