METABOLIC ALTERATIONS IN CANCER CELLS

Cancer: a metabolic disease?
METABOLIC CHARACTERISTICS OF CANCER CELLS

Increased GLYCOLYTIC ACTIVITY (Warburg Effect)
Increased production of LACTATE

Loss of PASTEUR’S EFFECT
(Oxygen inhibition of glycolysis)

INCREASED CONSUMPTION of GLUTAMINE

INCREASED PROTEIN SYNTHESIS

DECREASED PROTEOLYSIS

DECREASED SYNTHESIS OF FATTY ACIDS (increased lipolysis from host adipose tissue)
ENERGETIC METABOLISM IN TUMOURS

• WARBURG EFFECT
• In the 1920s, Otto Warburg observed that tumor cells consume a large amount of glucose, much more than normal cells, and convert most of it to lactic acid. This phenomenon, now known as the ‘Warburg effect,’ is the foundation of one of the earliest general concepts of cancer: that a fundamental disturbance of cellular metabolic activity is at the root of tumor formation and growth.
Biography

Otto Heinrich Warburg:
• Born-October 8, 1883 in Germany
• Died-August 1, 1970 in Berlin, Germany
• Son of physicist Emil Warburg
• Otto was a German physiologist and medical doctor.
• He won a Nobel prize in Physiology and Medicine for his Warburg effect in 1931.
• He was one of the twentieth century's leading biochemist
One of his Lectures...

- "Cancer, above all other diseases, has countless secondary causes. But, even for cancer, there is only one prime cause. Summarized in a few words, the prime cause of cancer is the replacement of the respiration of oxygen in normal body cells by a fermentation of sugar... " -- Dr. Otto H. Warburg in Lecture
The Warburg hypothesis

The prime cause of cancer is the replacement of the respiration of oxygen...by a fermentation of sugar..."
The Warburg Effect

- Pyruvate is an end-product of glycolysis, and is oxidized within the mitochondria.
- According to Warburg, cancer should be interpreted as a mitochondrial dysfunction.
- As a consequence, most cancer cells predominantly produce energy by a high rate of glycolysis followed by lactic acid fermentation in the cytosol, rather than by a comparatively low rate of glycolysis followed by oxidation of pyruvate in mitochondria like most normal cells. The latter process is aerobic. Tumour cells typically have glycolytic rates that are up to 200 times higher than those of their normal tissues of origin; this occurs even if oxygen is plentiful.
- He postulated that this change in metabolism is the fundamental cause of cancer.
Metabolism and patient outcome

Gene Set Enrichment Analysis

Metabolic Pathways linked to patient survival

Low Survival

High Survival

Survival

Metabolism and patient outcome
Metabolism and patient outcome

OXPHOS is suppressed in low survival patients

![Bar graph showing pathway enrichment in low vs high survival patients]

- OXPHOS
- Purine biosynthesis
- CYP metabolism
- FAO (mitochondrial)
- FAO (peroxisomal)
- Glycolysis and gluconeogenesis

Pathway enrichment low vs high survival
(Number of cancer types)
Evidence from *in vivo* experiments

Primary and metastatic brain tumors

NSCLC tumors

ccRCC tumors

↑↑ Glucose

↓ Pyruvate

↓ Lactate

↑↑ Glucose

↓ Pyruvate

→ TCA

↓ Lactate

Cell Metabolism Volume 28, Issue 5, (November 2018)
Aerobic glycolysis in cancer
detection of cancer exploiting glucose addiction

$[^{18}F]$fluoro-2-deoxyglucose (FDG) Positron Emission Tomography (PET)
FDG-PET shows increase in glucose uptake in cancer

https://en.wikipedia.org/wiki/Positron_emission_tomography
State-of-the-art imaging of cancer metabolism

Magnetic resonance imaging of tumor glycolysis using hyperpolarized $^{13}$C-labeled glucose

Tiago B Rodrigues$^1$, Eva M Serra$^1$, Brett W C Kennedy$^2$, De-En Hu$^2$, Mikko I Kettunen$^{1-3}$ & Kevin M Brindle$^{1-3}$
State-of-the-art imaging of cancer metabolism

Magnetic resonance imaging of pH in vivo using hyperpolarized $^{13}$C-labelled bicarbonate

Ferdia A. Gallagher$^{1,2,3,*}$, Mikko I. Kettunen$^{1,2,*}$, Sam E. Day$^{1,2,+}$, De-En Hu$^{1,2}$, Jan Henrik Ardenkjær-Larsen$^{4}$, René in ‘t Zandt$^{5}$, Pernille R. Jensen$^{5}$, Magnus Karlsson$^{5}$, Klaes Golman$^{5}$, Mathilde H. Lerche$^{5}$ & Kevin M. Brindle$^{1,2}$
there is an advantage to oxidative metabolism during nutrient limitation and to nonoxidative metabolism during cell proliferation
Aerobic glycolysis supports proliferation providing substrates for neosynthesis.
Schematic representation of the differences between oxidative phosphorylation, anaerobic glycolysis, and aerobic glycolysis (Warburg effect)

In proliferating cells, ~10% of the glucose is diverted into biosynthetic pathways upstream of pyruvate production. Cancer cells consume approx 200x glucose with respect to normal quiescent cells.
Aerobic glycolysis and “stemness”

<table>
<thead>
<tr>
<th>Stem cell marker</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Differentiation marker</td>
<td>Negative</td>
<td>Positive</td>
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</table>

Glycolysis

OXPHOS

Nature Reviews | Molecular Cell Biology
Cancer stem cells and plasticity
The Warburg effect describes the enhanced conversion of glucose to lactate by tumor cells, even in the presence of adequate oxygen. Activation of the AKT oncogene results in increased glucose transportation and stimulation of HK2 activity, which enhances glycolytic rates.

MYC oncogene activates glycolytic genes and mitochondrial biogenesis, which can result in ROS production. ROS could, in turn, cause mtDNA mutations that render mitochondria dysfunctional.

P53 stimulates respiration through activation of a component of the respiratory chain. HIF-1, increased by RAS, transactivates glycolytic genes as well as directly activates the PDK1 gene, which in turn inhibits PyruvateDH. (PDH catalyzes the conversion of pyruvate to acetyl-CoA, which enters the TCA cycle)

Inhibition of PDH by PDK1 attenuates mitochondrial function, resulting in the shunting of pyruvate to lactate.
CYCLE OF CORI: lactate is used for gluconeogenesis in Liver to support glucose demand.
CORI’s CYCLE: Liver gluconeogenesis from Lactate (along with glicogenolysis) provides glucose to cancer cells
LIVER metabolism provides glutamine to cancer cells
AMINOACID AVAILABILITY CONTROL PROTEIN SYNTHESIS

Amino Acids \(\downarrow\)

- uncharged tRNA \(\uparrow\)
- GCN2 \(\rightarrow\) GCN2(p)
- eIF2\(\alpha\)(p)
- mTOR inactive
  - Inhibits global protein synthesis
  - eIF4E-BP1
    - Proein Synthesis \(\downarrow\)

Amino Acids \(\uparrow\)

- uncharged tRNA \(\downarrow\)
- Cytoplasmic sensor?
- GCN2 \(\rightarrow\) GCN2(p)
- eIF2\(\alpha\)(p)
- Protein Synthesis \(\uparrow\)
- mTOR active
  - eIF4E-BP1
    - Proein Synthesis \(\uparrow\)
## Essential vs. non-essential amino acids

<table>
<thead>
<tr>
<th>Essential</th>
<th>Conditionally Non-Essential</th>
<th>Non-Essential</th>
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<tbody>
<tr>
<td>Histidine</td>
<td>Arginine</td>
<td>Alanine</td>
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<tr>
<td>Isoleucine</td>
<td>Asparagine</td>
<td>Aspartate</td>
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<td>Leucine</td>
<td>Glutamine</td>
<td>Cysteine</td>
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<td>Methionine</td>
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<td>Phenylalanine</td>
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<td>Threonine</td>
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<tr>
<td>Tryptophan</td>
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<td>Valine</td>
<td></td>
<td></td>
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<tr>
<td>Lysine</td>
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</table>
GLUTAMINE METABOLISM IN CANCER CELLS
Leucine → SLC7A5 → mTORC1 activation → Metabolic transformation → Protein translation → Cell proliferation

Glutamine → Nitrogen donor → Nucleotide and amino acid synthesis

Glutaminolysis → GLS → GSH → Glutamate → GLUD → Citrate → OAA → Malate → Succinate → Fumarate → Mitochondrion

Maintain redox balance

DNA aberrations → Histone methylation

Pyruvate → LDH → Lactate

Glucose → GLUT → Glucose

SLC1A5 → Leucine
Cancer cells require both energy and material to survive and duplicate in a competitive environment. Nutrients, such as amino acids (AAs), are not only a caloric source, but can also modulate cell metabolism and modify hormone homeostasis. Our hypothesis is that the environmental messages provided by AAs rule the dynamics of cancer cell life or death, and the alteration of the balance between essential amino acids (EAAs) and non-essential amino acids (NEAAs) (lower and higher than 50%, respectively) present in nutrients may represent a key instrument to alter environment-dependent messages, thus mastering cancer cells destiny. In this study, two AA mixtures, one exclusively consisting of EAAs and the other consisting of 85% EAAs and 15% NEAAs, were tested to explore their effects on the viability of both normal and cancer cell lines and to clarify the molecular mechanisms involved. Both mixtures exerted a cell-dependent anti-proliferative, cytotoxic effect involving the inhibition of proteasome activity and the consequent activation of autophagy and apoptosis. These results, besides further validating the notion of the peculiar interdependence and extensive crosstalk between the ubiquitin–proteasome system (UPS) and autophagy, indicate that variation in the ratio of EAAs and NEAAs can deeply influence cancer cell survival. Consequently, customization of dietary ratios among EAAs and NEAAs by specific AA mixtures may represent a promising anticancer strategy able to selectively induce death of cancer cells through the induction of apoptosis via both UPS inhibition and autophagy activation.
Tumor suppressors and proto-oncogenes regulate the metabolic pathways involved in tumor growth.
Cancer cells utilize GLUTAMINE as an energetic source in the TCA cycle.
Rol of the tumor microenvironment in the regulation of cancer cell metabolism. (A) Tumor cells, under hypoxic conditions, secrete lactate via MCT4. In response, cancer-associated fibroblasts (CAFs) and oxygenated tumor cells take up the tumor-extruded lactate. (B) Cancer cells induce ROS production in CAFs, leading to the onset of stromal oxidative stress, which in turn, drives autophagy and provides recycled nutrients via catabolism and aerobic glycolysis to feed the appetite of adjacent cancer cells. (c) Tumor stromal cells are able to take up cystine, convert it to the amino acid cysteine, and then secrete it. Tumor cells then use cysteine to produce glutathione, resulting in increased ROS resistance and survival. (D) Adipocytes provide tumor cells with fatty acids supplying the energy needs of rapid tumor growth. (E) Glutamine can be hydrolyzed as ammonia in tumor cells and reused by CAFs. (F) CAFs secrete glutamine into the tumor microenvironment to meet the glutamine needs of the cancer cells. MCT4, monocarboxylate transporter 4; GLUT1: Glucose transporter 1; ASC: Neutral amino acid transporter A; XC^-: Cystine/glutamate transporter; ROS: Reactive Oxygen Species; OAA: Oxaloacetate.
“The prime cause of cancer is the replacement of the respiration of oxygen ... in normal body cells by fermentation of sugar”

Otto Warburg 1956

Tumor cells derive nearly all of their fatty acids from de novo synthesis

Sidney Weinhouse 1953
remember citrate lyase?
FA synthesis is reduced in glycolytic tumor cells
Cancer anabolic metabolism inhibitors move into clinic
<table>
<thead>
<tr>
<th>Company/location</th>
<th>Agent</th>
<th>Target</th>
<th>Indications</th>
<th>Status</th>
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</thead>
<tbody>
<tr>
<td>Agios &amp; Celgene</td>
<td>AG-221, AG-120, AG-881</td>
<td>IDH1 and IDH2</td>
<td>AML, MDS, solid tumors</td>
<td>Phase 3</td>
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<tr>
<td>Polaris Group/San Diego</td>
<td>ADI-PEG 20</td>
<td>Pegylated arginine deiminase</td>
<td>Hepatocellular carcinoma (HCC), others</td>
<td>Phase 3 in HCC missed primary endpoint</td>
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<td>Cornerstone Pharmaceuticals/Cranbury, New Jersey</td>
<td>CPI-613</td>
<td>Pyruvate dehydrogenase</td>
<td>AML, MDS, solid tumors</td>
<td>Phase 2</td>
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<td>Calithera Biosciences</td>
<td>CB-839</td>
<td>Glutaminase 1</td>
<td>RCC, breast cancer</td>
<td>Phase 1/2</td>
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<td>3-V Biosciences</td>
<td>TVB-2640</td>
<td>Fatty acid synthase</td>
<td>Ovarian, breast, lung</td>
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<td>IDH305</td>
<td>IDH1</td>
<td>Advanced cancers</td>
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<td>IDH1</td>
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<td>Bayer/Leverkusen, Germany</td>
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<td>Advanced Cancer Therapeutics/Louisville, Kentucky</td>
<td>PFK-158</td>
<td>PFKFB3</td>
<td>Solid tumors</td>
<td>Phase 1</td>
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<td>Aeglea BioTherapeutics/Austin, Texas</td>
<td>AEB-1102</td>
<td>Modified human arginase</td>
<td>AML, MDS, solid tumors</td>
<td>Phase 1</td>
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</tbody>
</table>
Resistance through metabolic reprogramming
Metabolism of cancer cells is different from that of normal cells.

Dysregulated metabolism can drive oncogenic processes.

Altered metabolism offers a therapeutic window to target cancer cells.
Key references & links

- **JS. Flier et al.** Elevated levels of glucose transport and transporter messenger RNA are induced by ras or src oncogenes. *Science*, 1987
- **Pavlova and Thompson**, The emerging hallmarks of cancer metabolism, *Cell Metabolism*, 2016
- **Sanderson et al**, Revisiting the Warburg Effect: Some Tumors Hold Their Breath, *Cell Metabolism* 2018

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