Global profiling strategies for mapping dysregulated metabolic pathways in cancer
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How cancer metabolism is tuned for proliferation and vulnerable to disruption
A Schulze & AL Harris
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The ‘big’ picture

[Diagram of metabolic pathways, including glycogen metabolism, pentose phosphate pathway, sorine synthesis, pH regulation, and fatty acid synthesis.]
The Warburg effect ->
- Intracellular acidification
- Lactate accumulation.

Maintaining an alkaline intracellular milieu is essential for cell survival.

Acidification of the extracellular micro-environment may facilitate invasion and metastasis formation.

Main acid-regulatory systems
- Na+/H+ exchangers
- Carbonic anhydrase 9 (CA9)
- Monocarboxylate transporters (MCT).
Cells within oxygenated areas of tumors may use lactate produced by hypoxic tumor cells. This use increases glucose availability and supports the survival of cells within the hypoxic part of the tumor.

*Recommended: Ben-Jacob et al., Trends in Microbiology, 2012*
The role of TCA enzymes in cancer

- Inherited mutations in succinate dehydrogenase (SDH) or fumarate hydratase (FH) -> familial cancer syndromes.
- Inhibition of SDH or FH -> accumulation of succinate or fumarate
  - -> Inhibition of prolyl hydroxylases (enable HIF degradation) -> accumulation of HIF
  - -> Inhibition of other α-ketoglutarate-dependent dioxygenases (histone and DNA demethylases) -> epigenetic alterations.
1. Mutations in the active site at a single amino acid residue of the enzyme IDH1/2 are a common in low-grade gliomas, secondary glioblastomas and leukemic cancers.
2. This pattern indicated a gain-of-function mutations.
3. Glioblastoma cells transfected with mutant IDH1 have an increase in 2-hydroxyglutarate (2-HG). (Dang et al., Nature, 2009)
4. Mutant IDH1 consumed NADPH and reduced a-ketoglutarate to 2-HG. (Dang et al., Nature, 2009)

* 2-HG can be formed in cells possessing wild-type IDH1 and IDH2.
Both mutation in IDH1 and separate treatment with 2-HG resulted in a host of downstream metabolic changes involving:

- Amino acids
- GSH metabolites
- Choline derivatives
- TCA cycle intermediates.
The epigenetic effects of 2-HG

- IDH1/2 mutations increase CpG island methylation and promoter hypermethylation.
- 2-HG can act as a competitive inhibitor of α-ketoglutarate-dependent demethylases as histone demethylases.
Accumulation of 2-HG is cancerous also in inborn errors of metabolism

- (L)-2-hydroxyglutaric aciduria - an autosomal-recessive condition caused by deficiency of (L)-2-HG dehydrogenase.
  \[ 2\text{-HG} \rightarrow \alpha\text{-ketoglutarate} \]
- Cause the accumulation of 2-HG in body fluids.
- Many children with this disease develop brain tumors or other types of cancer.
Reductive carboxylation

- Is triggered by
  - Hypoxia
  - Mitochondrial dysfunction
  - Deficiency in the von Hippel-Lindau (VHL) tumor suppressor
- Knocking down IDH2 impairs cell proliferation.
Cancer cells must increase fatty acids production in one of three ways:

- Endogenous production (via FASN)
- Release of esterified fatty acids (via MAGL)
- Release and absorption of fatty acid sources.
Unsaturated fatty acids and membrane fluidity

- Unsaturated fatty-acids are required to maintain membrane fluidity.
- If cancer cells endogenously produce fatty-acids they need to desaturate them.
- Fatty-acid desaturation requires oxygen.
- The effects of fatty-acid deprivation are more pronounced under hypoxia.
In ovarian cancer (Neiman et al., *Nature Medicine*, 2011)

- Adipocytes promote growth and metastasis formation and provide the cancer lipids.
- Fatty acid binding protein 4 (FABP4) is upregulated in metastases as compared to primary tumors.
- FABP4 deficiency impairs metastasis in mice.
Serine and glycin metabolism – the role of PHGDH

- In some cancer cells glycolytic carbon is largely diverted into serine and glycine metabolism.
- Phosphoglycerate dehydrogenase (PHGDH) catalyzes the first committing step in this pathway.
- PHGDH is frequently amplified in cancer.
- PHGDH protein levels are elevated in 70% of estrogen receptor negative breast cancers.
PHGDH knockdown decreases
- The level of TCA cycle intermediates
- Anaplerosis of glutamine to α-ketoglutarate.

In these breast cancer cells serine synthesis pathway is responsible for ~50% of the net conversion of glutamine to α-ketoglutarate.
Glycine consumption and cancer cell proliferation

- A highly parallel metabolomics approach established cellular consumption and secretion profiles of more than 200 metabolites across the NCI-60 panel (Jain et al., Science 2012).
- Glycine consumption correlates with cellular proliferation rate.
- Targeting glycine metabolism could selectively compromise nucleotide biosynthesis in rapidly proliferating cancer cells.
PKM2 provides a selective growth advantage for tumor cells

- PKM2 is highly expressed in rapidly proliferating tissues.
- Many cancer cells exclusively express this isoform.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>PKM₁</th>
<th>PKM₂</th>
</tr>
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<tbody>
<tr>
<td>Oxygen consumption</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>Lactate production</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td>Tumor aggressiveness</td>
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<td>↑</td>
</tr>
<tr>
<td>Active</td>
<td>Always</td>
<td>Sometimes</td>
</tr>
<tr>
<td>Posttranscriptional regulation</td>
<td>No</td>
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</table>
PKM2 post-transcriptional regulation

- PKM2 can switch from a tetrameric to a dimeric form with lower activity.
- PKM2 is regulated by
  - Tyrosine kinase signaling
  - Oxidative stress
  - Acetylation
  - Serine
Noncatalytic functions of PKM2

- Epidermal growth factor receptor (EGFR) activation induces nuclear translocation of PKM₂.
- Its translocation initiates cyclin D₁ expression, which is fundamental to cell proliferation and brain tumor development.
- PKM₂ interacts directly with the HIF-1α subunit and promotes transactivation of HIF-1 target genes.
**PKM2 as a therapeutic target**

- **Activators of PKM2**
  - Quantitative screening of ~300,000 small molecules using an ATP-generation assay coupled to luminescence.
  - One of the identified molecules enhances cancer cell death under oxidative stress.

- Downregulate PKM2 expression to impair its noncatalytic protein-protein interactions.
Activation of HIF due to anti-angiogenic drugs

- Decreases mitochondrial oxidation of pyruvate by inducing PDHK1
- Increases pyruvate conversion to lactate by inducing LDHA expression
- Regulates intracellular pH by inducing CA9.

Combining anti-angiogenic drugs with inhibitors of HIF or inhibitors of its downstream targets blocks the adaptive response to hypoxia.
Cells adopt to metformin and antimetabolites by inducing glucose uptake and glycolysis.

Combination of metformin or antimetabolites with the glycolysis inhibitor 2-deoxyglucose (2-DG) prevents the metabolic adaptation to AMPK activation.
Some of the metabolic phenotypes of cancer are similar to those of healthy proliferating cells.
Which metabolic alterations are cancer-specific?
What kind of metabolic interactions occur between the different cells in the tumor?
What are the changes that occur beyond central metabolism? (e.g., detoxification of drugs)
The interplay between metabolism and other cellular processes in cancer.
Questions?