Evidence for an Alternative Glycolytic Pathway in Rapidly Proliferating Cells

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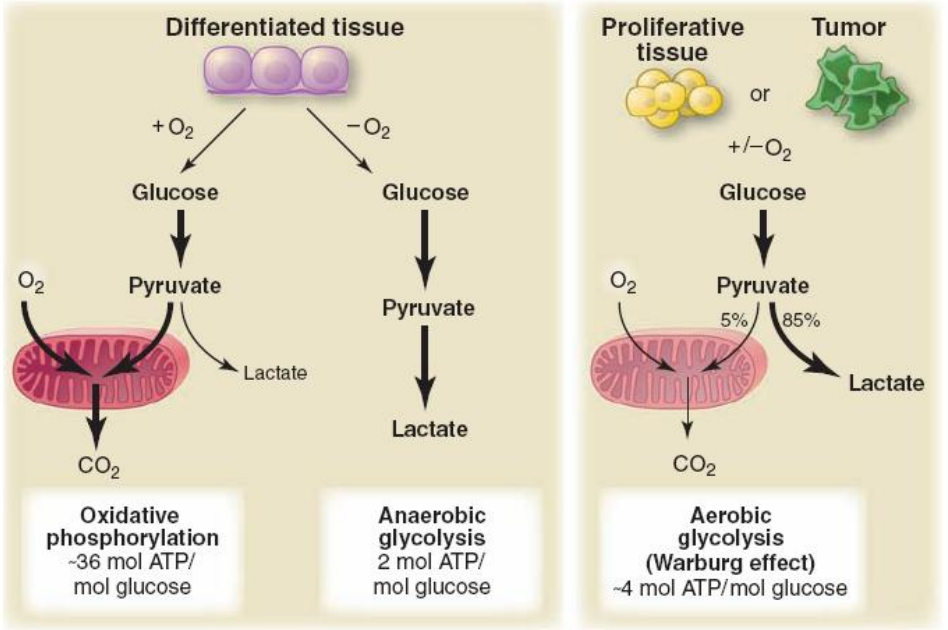
Introduction

The Warburg Effect

- Cancer cells metabolize glucose differently
  - Primarily by glycolysis instead of oxidative phosphorylation
  - Leads to secretion of non-oxidized carbons in the form of lactate



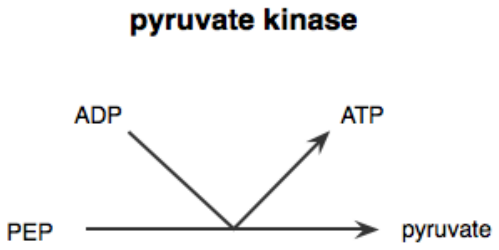
Metabolic Influence of Pyruvate Kinase

- The glycolytic enzyme pyruvate kinase is alternatively spliced to two isoforms: PKM1, PKM2

- The expressed isoform influences the metabolism of glucose

  - Cells expressing PKM2 produce more lactate and consume less oxygen than cells expressing PKM1 (Christofk et al. Nature 2008)

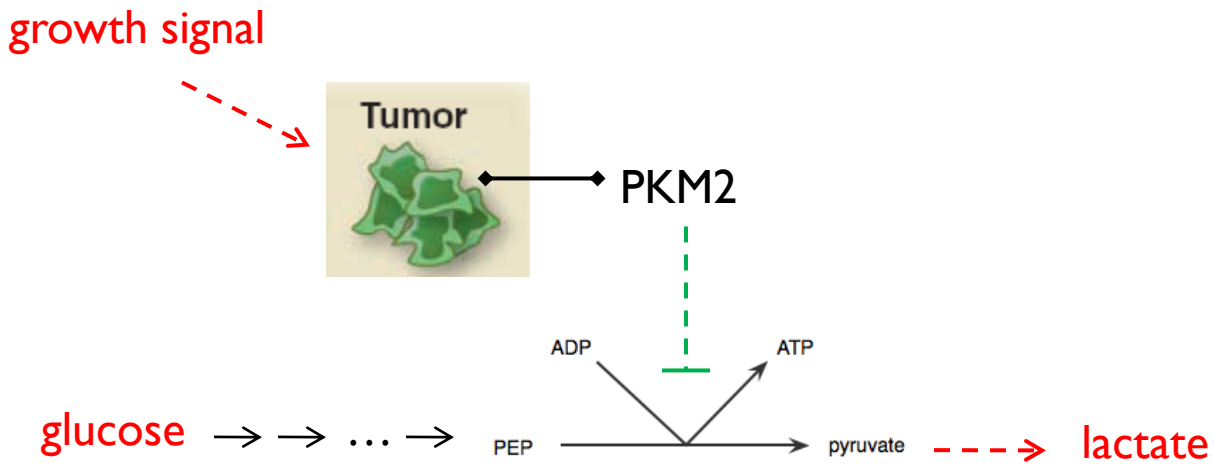
  - All cancer cells studied to date exclusively express PKM2, whereas cells in many normal differentiated tissues express PKM1



Metabolic Influence of Pyruvate Kinase

- PKM2 differs from PKM1 in that its activity can be negatively regulated in response to growth factor signaling by binding to tyrosine-phosphorylated proteins

  - This ability appears to be important for cell proliferation (Christofk et al. Nature 2008)



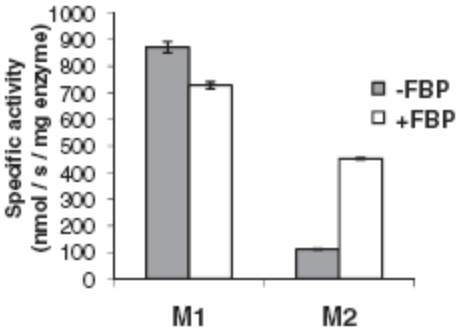
Metabolic Influence of Pyruvate Kinase

- Complete catabolism of pyruvate to CO_2 may be deleterious in dividing cells
  - may limit the availability of precursors and reducing potential necessary to produce biomass
- Pentose phosphate pathway, an alternative pathway of glycolysis, produces:
  - NADPH: reductive biosynthesis reactions within cells. (e.g. fatty acid synthesis)
  - Ribose-5-phosphate (R5P): synthesis of nucleotides and nucleic acids.
  - Erythrose-4-phosphate (E4P): synthesis of aromatic amino acids, precursors for many biosynthetic pathways.

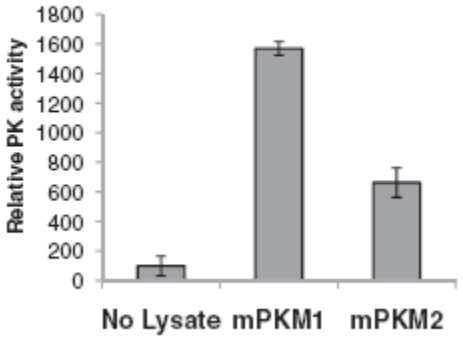
Results

PKM2 is less active than PKM1 in vitro and in cells

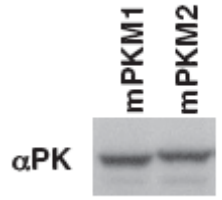
- In vitro: the specific activity of PKM2 is substantially lower than of PKM1



- In vivo: PKM2-expressing cells exhibited less than half the pyruvate kinase activity of cells expressing the equivalent amount of PKM1

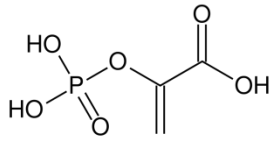


- Under these identical conditions, PKM2 expression provides a selective advantage for growth in vivo (Christofk et al. Nature 2008)



Donation of phosphate from PEP to PGAM1

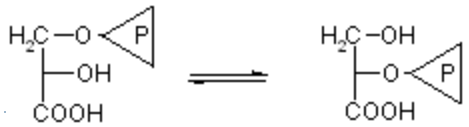
- The authors hypothesized that PEP (a substrate of pyruvate kinase) possibly transfers its phosphate to a protein in mammalian cells



  - Bacteria use PEP as the initial phosphate donor for protein phosphorylation in a signaling cascade that regulates carbohydrate metabolism in response to nutrient availability

  - Plants: transfer of the PEP phosphate to a protein occurs as an enzymatic intermediate within the Calvin cycle

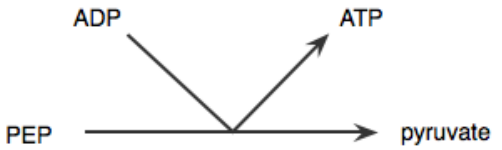
- Identified such cytosolic protein as phosphoglycerate mutase (PGAM1)



3-Phosphoglycerate 2-Phosphoglycerate

Association of PGAM1 phosphorylation with pyruvate generation from PEP in the absence of pyruvate kinase

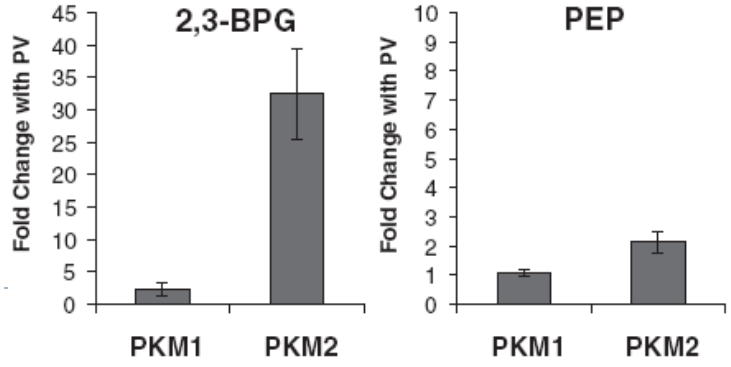
- Knockdown of pyruvate kinase resulted in enhanced PEP-phosphorylated PGAM1



- Switching cells from PKM2 to PKM1 expression reduced the amount of phosphorylated PGAM1

- Acute inhibition of PKM2 led to:

PGAM1:
3PG + Enzyme-P → [Enzyme-2,3BPG] →
2PG + Enzyme-P

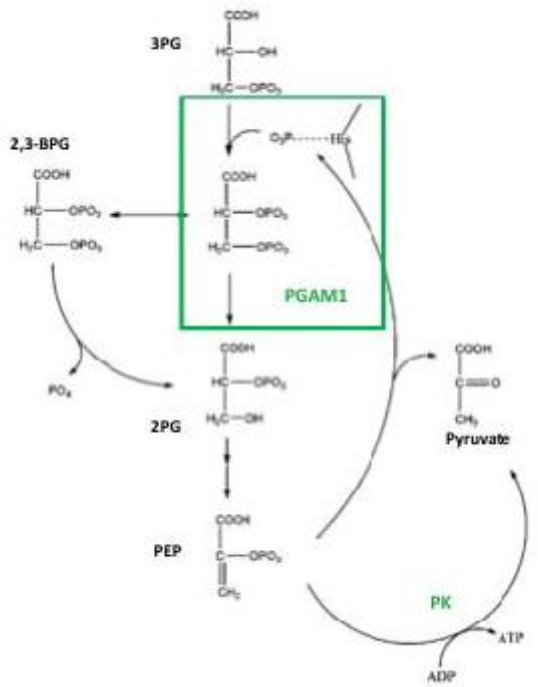


Association of PGAM1 phosphorylation with pyruvate generation from PEP in the absence of pyruvate kinase

- PGAM1-phosphorylating activity caused generation to pyruvate from PEP in the absence of pyruvate kinase
  - Alternative pathway to generate pyruvate

- Cells expressing PKM2 → lower activity of pyruvate kinase → higher phosphorylation of PGAM1 → pyruvate is generated through alternative pathway

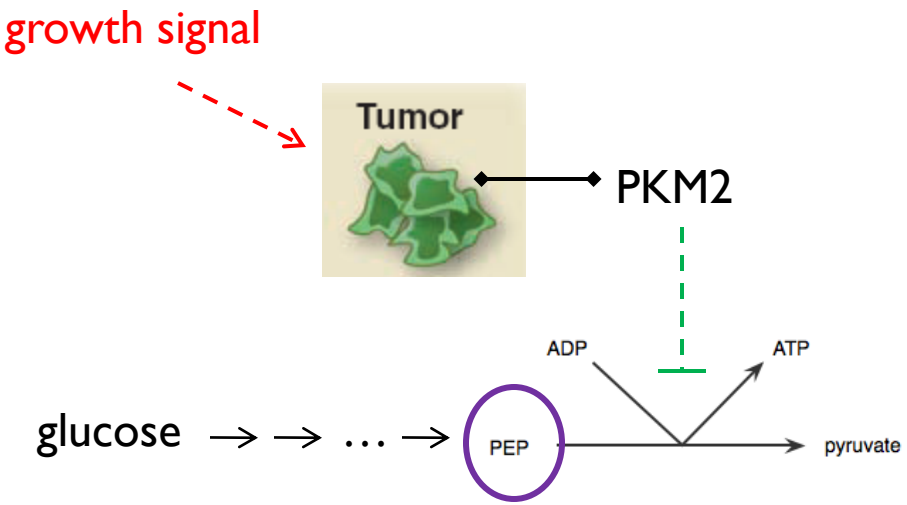
- Does the alternative pathway provide an advantage to proliferating cells?

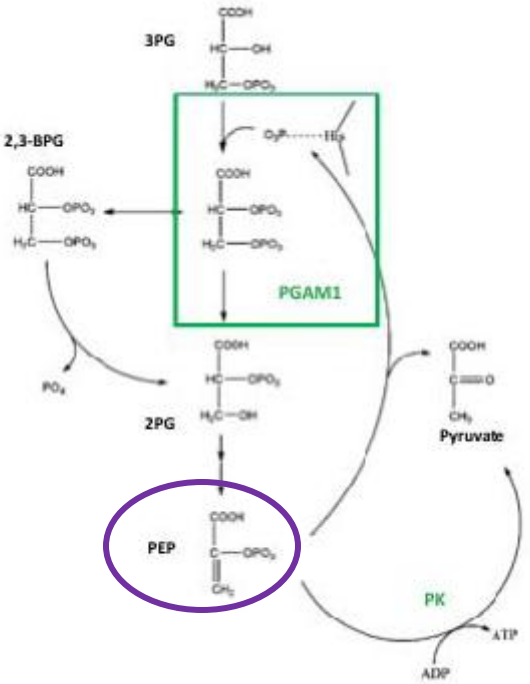


Discussion

Discussion

- One important consequence of down-regulating PKM2 activity by tyrosine kinases may be to increase H11-phosphorylated PGAM1





Discussion

- Phosphorylation of PGAM1 increases the mutase function of the enzyme
  - 3PG + Enzyme-P → [Enzyme-2,3BPG] → 2PG + Enzyme-P

- This generates a positive feedback loop
  - production of PEP increases (2PG is its precursor)
    ➔ the enzymatic activity of PGAM1 increases

  - may promote the redistribution of metabolites upstream of PGAM1 into biosynthetic pathways that branch from glycolysis

Discussion

- The authors propose an alternate glycolytic pathway that convert PEP into pyruvate
  - In the absence of pyruvate kinase, its rate is comparable to its Vmax
- May explain how cancer cells with less pyruvate kinase activity continue to display a high rate of glycolysis
- This pathway provides an advantage to proliferating cells as it is not coupled to ATP synthesis
  - Excess ATP allosterically inhibit PFK and other rate-limiting steps in glycolysis

Discussion

- PGAM1 may have a previously unappreciated regulatory function in controlling glycolysis in proliferating cells
  - differential phosphorylation of PGAM1 in PKM2- versus PKM1-expressing cells and tissues
- PGAM1 is unique among the glycolytic enzymes
  - its transcription is regulated by the tumor suppressor p53
  - increased expression of PGAM1 has been reported to immortalize primary cells through an unknown mechanism
- PGAM1 was also identified as the target of a compound from a chemical genomics screen for molecules that inhibit breast cancer cell growth

THANKS