

# **Evidence for an Alternative Glycolytic Pathway in Rapidly Proliferating Cells**

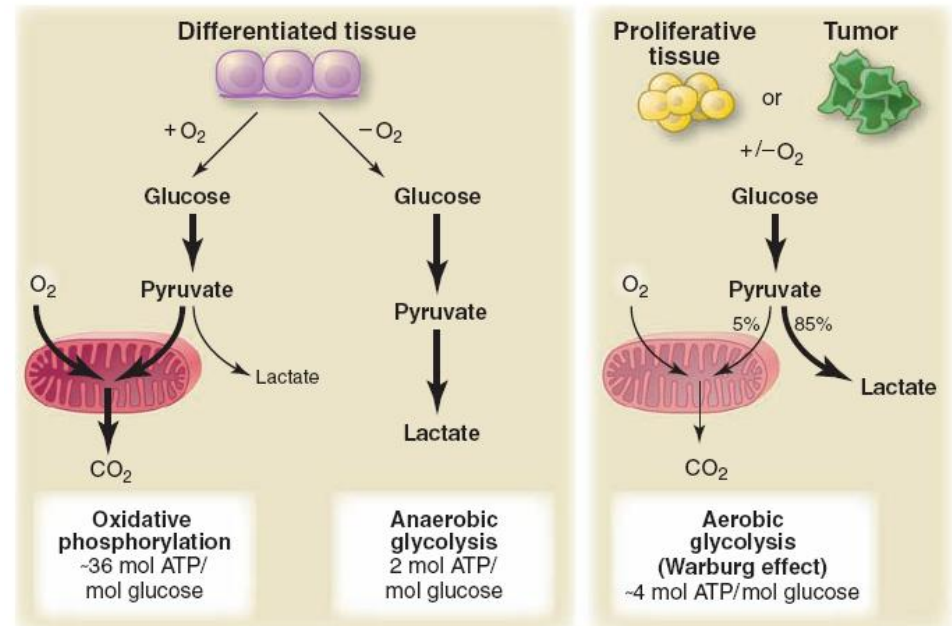
Matthew G. Vander Heiden, *et al.*

*Science* **2010**

# Introduction

# The Warburg Effect

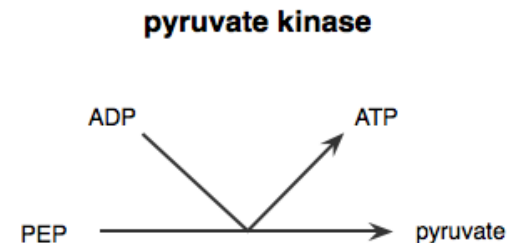
- ▶ Cancer cells metabolize glucose differently
  - ▶ Primarily by glycolysis instead of oxidative phosphorylation
  - ▶ Leads to secretion of non-oxidized carbons in the form of lactate



# Metabolic Influence of Pyruvate Kinase

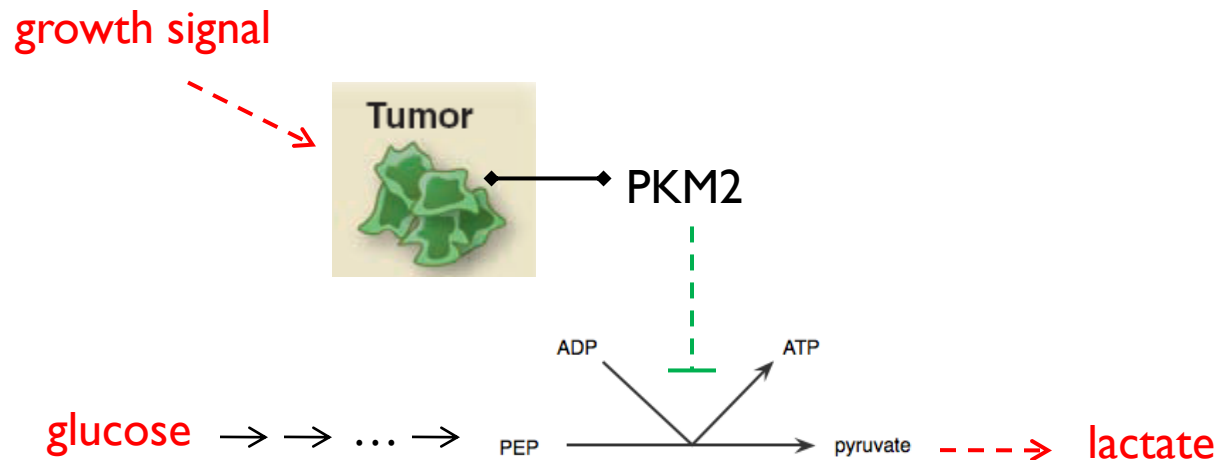
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- ▶ The glycolytic enzyme **pyruvate kinase** is alternatively spliced to two isoforms: **PKM1, PKM2**
- ▶ The expressed isoform influences the metabolism of **glucose**
- ▶ Cells expressing **PKM2** produce more **lactate** and consume less **oxygen** 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

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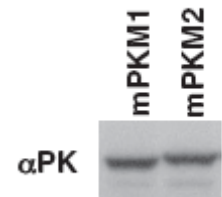
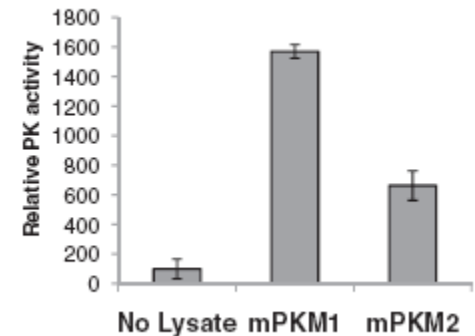
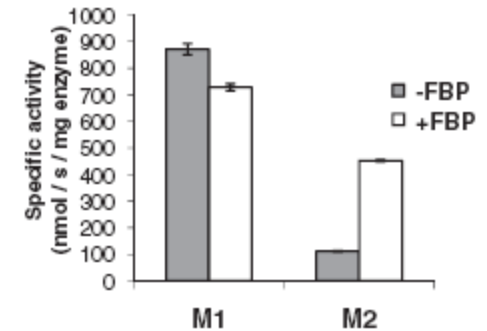
- ▶ Complete catabolism of **pyruvate** to **CO<sub>2</sub>** may be deleterious in dividing cells
  - ▶ may limit the availability of precursors and reducing potential necessary to produce biomass
- ▶ **Pentose phosphate pathway**, an alternative pathway of glycolysis, produces:
  - ▶ **NADPH**: reductive 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

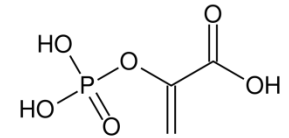
- ▶ In vitro: the specific activity of **PKM2** is substantially lower than of **PKM1**
- ▶ In vivo: **PKM2**-expressing cells exhibited less than half the **pyruvate kinase** activity of cells expressing the equivalent amount of **PKM1**
- ▶ Under these identical conditions, **PKM2** expression provides a selective advantage for growth in vivo (Christofk et al. Nature 2008)



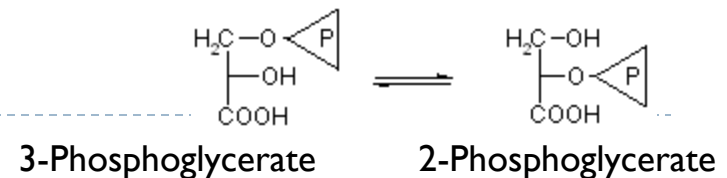


# Donation of phosphate from PEP to PGAM1

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

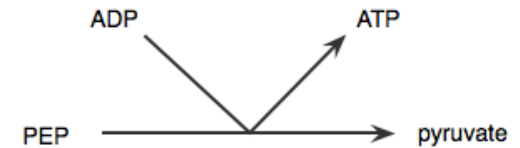


- ▶ Bacteria use PEP as the initial phosphate donor for protein phosphorylation in a signaling cascade that regulates carbohydrate metabolism in response to nutrient availability
- ▶ Plants: transfer of the PEP phosphate to a protein occurs as an enzymatic intermediate within the Calvin cycle
- ▶ Identified such cytosolic protein as phosphoglycerate mutase (PGAM1)



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

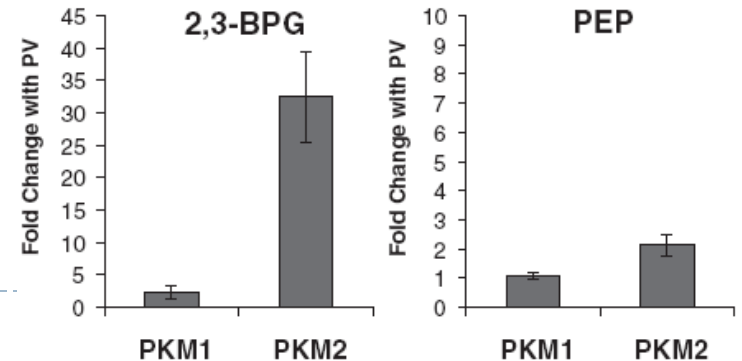
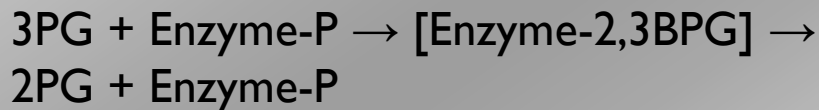
- ▶ Knockdown of **pyruvate kinase** resulted in enhanced PEP-phosphorylated **PGAM1**



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

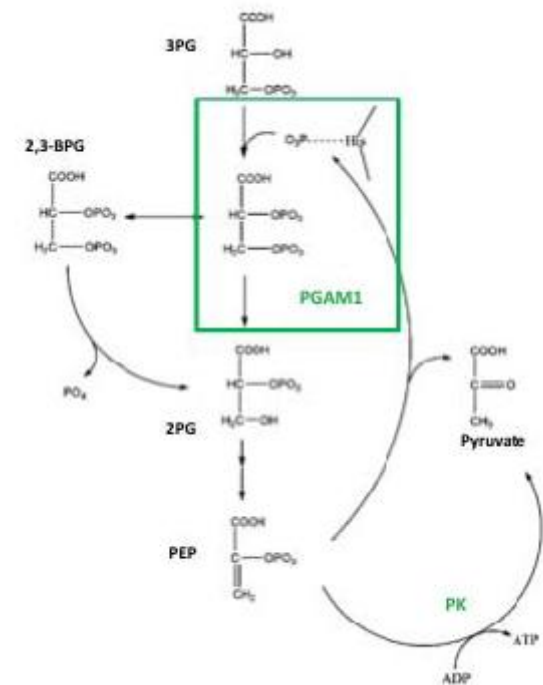
- ▶ Acute inhibition of **PKM2** led to:

## **PGAM1:**



# 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?

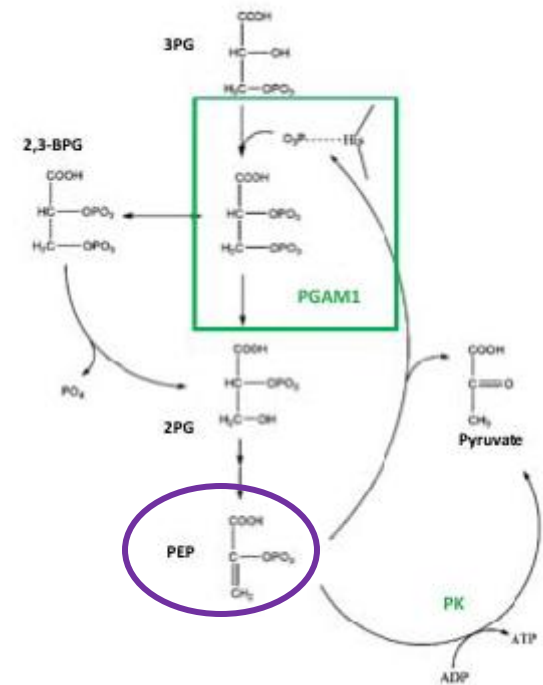
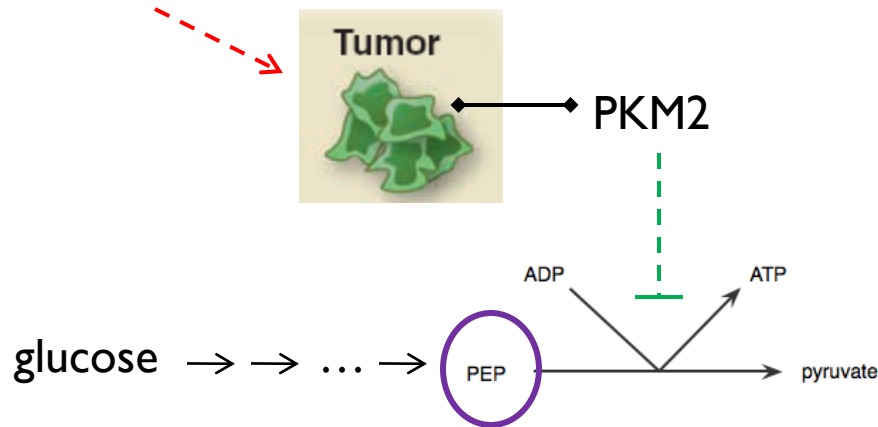


# Discussion

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- ▶ One important consequence of down-regulating **PKM2** activity by tyrosine kinases may be to increase H1I-phosphorylated **PGAM1**

growth signal



# Discussion

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- ▶ Phosphorylation of **PGAMI** increases the mutase function of the enzyme
  - ▶  $3\text{PG} + \text{Enzyme-P} \rightarrow [\text{Enzyme-2,3BPG}] \rightarrow 2\text{PG} + \text{Enzyme-P}$
- ▶ This generates a positive feedback loop
  - ▶ production of **PEP** increases (**2PG** is its precursor)
    - ➔ the enzymatic activity of **PGAMI** increases
  - ▶ may promote the redistribution of metabolites upstream of **PGAMI** into biosynthetic pathways that branch from glycolysis



# Discussion

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- ▶ The authors propose an alternate glycolytic pathway that convert **PEP** into **pyruvate**
  - ▶ In the absence of **pyruvate kinase**, its rate is comparable to its  $V_{max}$
- ▶ 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

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





# THANKS

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