

## Carbohydrate Metabolism: Regulation & Cell Biology

BIOCHEM 201 / INT 111

Professor S. Kim

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### Regulation of metabolic pathways

The flow of intermediates through metabolic pathways is controlled by four mechanisms:

- Substrate availability
- Allosteric activation or inhibition
- Covalent modification of enzymes
- Induction/repression of enzyme synthesis

Metabolic regulation in rapidly growing tissues is different from adult tissues.

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### Regulation of metabolic pathways in rapidly growing tissues

Metabolic regulation in rapidly growing tissues is different from adult tissues.

Cancer cells have altered metabolism:  
Otto Warburg (1929) observed that metabolic demand of tumor cells is significantly higher than most other tissues

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### Warburg effect

Increased glycolysis, even in the presence of oxygen, is a universal property of cancer and possibly its main cause.

Modern view of cancer is a disease of genes.

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### New evidence for Warburg effect



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### New evidence for Warburg effect

Perturbational profiling of a cell-line model for tumorigenesis by using metabolic measurements

*Ramanathan, A et al. PNAS 102, 5992*

We performed a three-dimensional screening experiment using 4 cell lines, 5 small-molecule perturbagens, and a large number of metabolic measurements. Analysis of these experiments provided insight into the relationship between two models of carcinogenesis, one (the Warburg hypothesis) based on increased energy production by glycolysis in cancer cells in response to aberrant respiration, and one based on cancer-causing genes. Rather than being opposing models, the approach described here suggests that these **two models are interlinked**. The cancer-causing genes used in this study appear to increase progressively the cell's dependence on glycolytic energy production and to decrease its dependence on mitochondrial energy production. However, mitochondrial biogenesis appears to have a more complex dependence, increasing to its greatest extent at an intermediate degree of transduction rather than at the fully transformed state.

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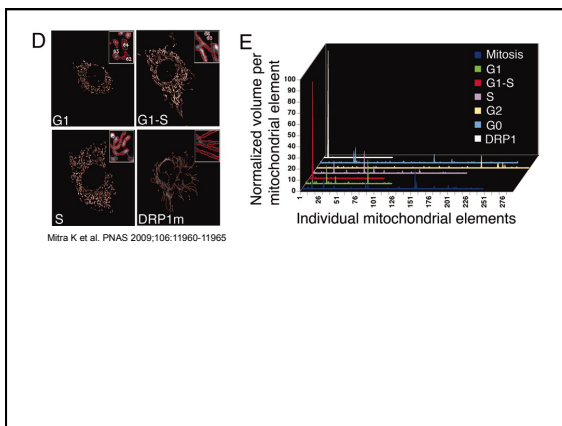
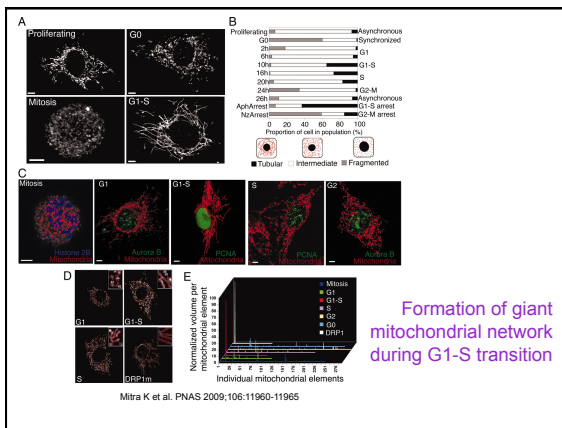
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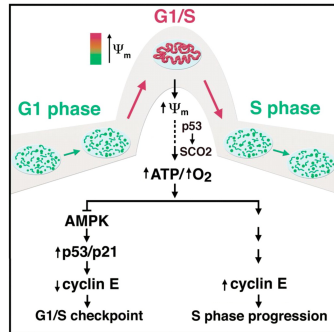
New evidence for Warburg effect

P53 regulates mitochondrial respiration  
 Matoba, S., et al. Science 312, 1650

The energy that sustains cancer cells is derived preferentially from glycolysis. This metabolic change, the Warburg effect, was one of the first alterations in cancer cells recognized as conferring a survival advantage. Here, we show that **p53, one of the most frequently mutated genes in cancers, modulates the balance between the utilization of respiratory and glycolytic pathways.** We identify Synthesis of Cytochrome c Oxidase 2 (SCO2) as the downstream mediator of this effect in mice and human cancer cell lines. SCO2 is critical for regulating the cytochrome c oxidase (COX) complex, the major site of oxygen utilization in the eukaryotic cell. Disruption of the SCO2 gene in human cancer cells with wild-type p53 recapitulated the metabolic switch toward glycolysis that is exhibited by p53-deficient cells. That SCO2 couples p53 to mitochondrial respiration provides a possible explanation for the Warburg effect and offers new clues as to how p53 might affect aging and metabolism.



Krebs cycle meets the cell cycle:  
mitochondria and the G1-S transition



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Warburg effect is still controversial

Is it a myth?

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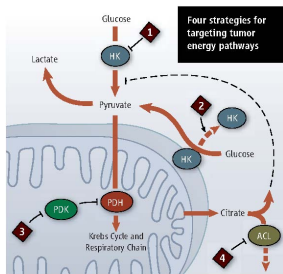
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Anti-mitotic targets  
within carbohydrate metabolic pathways



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## Pyruvate kinase M2 is a phosphotyrosine-binding protein

*Christofk, H., et al. Nature 452, 181*

Growth factors stimulate cells to take up excess nutrients and to use them for anabolic processes. The biochemical mechanism by which this is accomplished is not fully understood but it is initiated by phosphorylation of signalling proteins on tyrosine residues. 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 selectively to tyrosine-phosphorylated peptides. We show that binding of phosphotyrosine peptides to PKM2 results in release of the allosteric activator fructose-1,6-bisphosphate, leading to inhibition of PKM2 enzymatic activity. We also provide evidence that this regulation of PKM2 by phosphotyrosine signalling diverts glucose metabolites from energy production to anabolic processes when cells are stimulated by certain growth factors. Collectively, our results indicate that expression of this phosphotyrosine-binding form of pyruvate kinase is critical for rapid growth in cancer cells.

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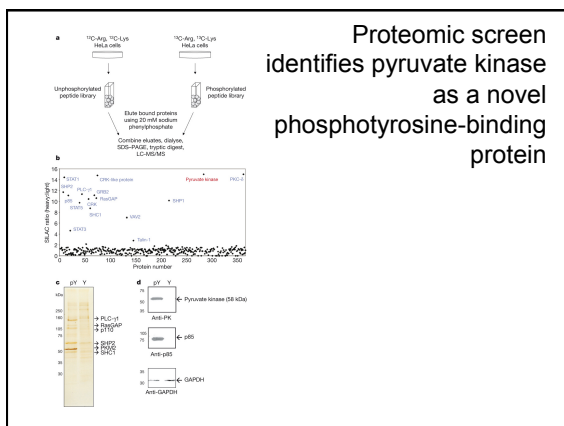
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## Proteomic screen identifies pyruvate kinase as a novel phosphotyrosine-binding protein




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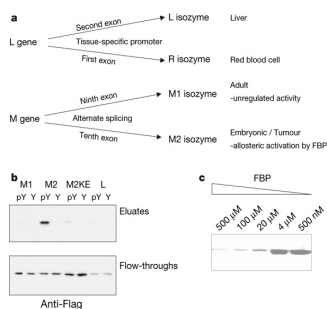
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Phosphopeptide binding is specific to the M2 isoform of pyruvate kinase and involves lysine 433 near the FBP binding pocket.

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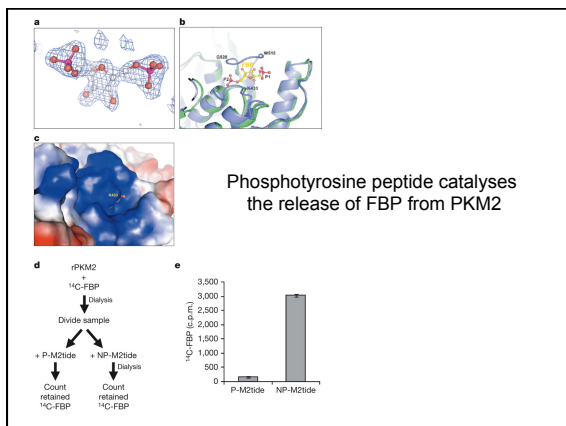
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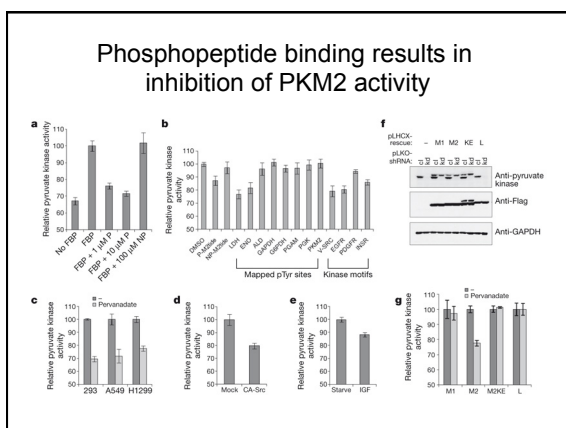
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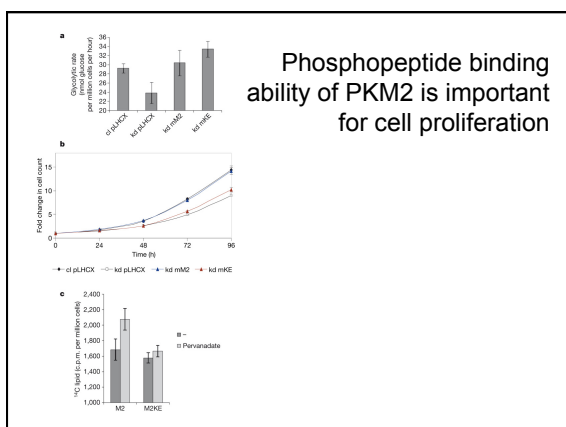
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### M2 splice isoform of pyruvate kinase is important for cancer metabolism and tumour growth

Christofk, H., et al. *Nature* 452, 230

Many tumour cells have elevated rates of glucose uptake but reduced rates of oxidative phosphorylation. How tumour cells establish this altered metabolic phenotype and whether it is essential for tumorigenesis is as yet unknown. Here we show that a single switch in a splice isoform of the glycolytic enzyme pyruvate kinase is necessary for the shift in cellular metabolism to aerobic glycolysis and that this promotes tumorigenesis. Tumour cells have been shown to express exclusively the embryonic M2 isoform of pyruvate kinase<sup>2</sup>. Here we use short hairpin RNA to knockdown pyruvate kinase M2 expression in human cancer cell lines and replace it with pyruvate kinase M1. Switching pyruvate kinase expression to the M1 (adult) isoform leads to reversal of the Warburg effect, as judged by reduced lactate production and increased oxygen consumption, and this correlates with a reduced ability to form tumours in nude mouse xenografts. These results demonstrate that M2 expression is necessary for aerobic glycolysis and that this metabolic phenotype provides a selective growth advantage for tumour cells *in vivo*.

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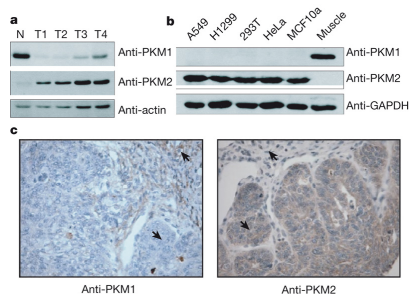
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### Tumour tissues and cell lines express the M2 isoform of pyruvate kinase




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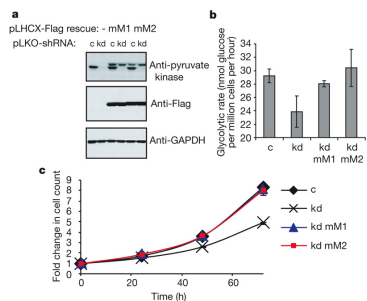
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### PKM2 knockdown is rescued by expression of PKM1 *in vitro*




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