

Glucose Metabolism in Cancer: The Saga of Pyruvate Kinase Continues

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Altered glucose metabolism is common in cancer. In this issue of *Cancer Cell*, Morita et al. report new mouse models that express specific isoforms of pyruvate kinase to study glycolysis in tumors. They report several unanticipated findings that challenge current ideas in cancer metabolism.

A common feature of cancer is the adaptation of cellular metabolism to satisfy the demands of inappropriate growth, survival, and proliferation. This is marked by the Warburg Effect, which is characterized by hyperactive glucose metabolism. The fitness advantage conferred to cells undergoing the Warburg Effect remains an active area of research (Liberti and Locasale, 2016). Numerous ongoing drug development efforts to target glucose metabolism are limited by our understanding of the cancer biology and the pharmacological principles needed to produce therapeutic outcomes.

One attractive approach to targeting glucose metabolism has been to exploit the differential expression of isoenzymes that are present throughout the glycolytic pathway, the most-studied among which are the isoforms of the pyruvate kinase M (Dayton et al., 2016). *PKM* encodes the enzyme pyruvate kinase, which catalyzes the final, irreversible step in glycolysis involving the transfer of a phosphate from phosphoenolpyruvate to ADP to generate pyruvate and ATP (Figure 1). There are two isoforms of PKM, which arise from alternative splicing resulting in mutually exclusive inclusion of nucleotides encoded by exon 9 or 10 of *PKM*, to give rise to PKM1 and PKM2, respectively. Interestingly, PKM1 and PKM2 differ in only 22 amino acids, but this allows for a host of properties that can affect glucose metabolism. PKM2, the less-active enzyme, can be negatively regulated by binding to phosphorylated tyrosine and reactive oxygen species and can be positively regulated by serine, the nucleotide intermediate SAICAR, and its classic activator fructose-1,6-bisphosphate (FBP). Notably, FBP is generated upstream in glycolysis, creating a

feedforward loop. Increases in FBP levels activate PKM2, and this mechanism allows cells to sense changes in glucose availability by increasing glycolytic rate (van Heerden et al., 2014). PKM1, the more-active enzyme, is notably lacking positive FBP regulation and can be perhaps thought of as a constitutively active version of PKM2.

The role of pyruvate kinase isoforms in cancer has a storied history. An original report using knockdown of the endogenous *PKM* gene products and ectopic re-expression of PKM1 or PKM2 cDNAs showed that PKM2 expression could promote metabolic adaptations to nutritional stress in cell culture and increase tumor growth in mouse xenografts (Christofk et al., 2008). The model that emerged from this and related studies was that PKM2, a less-active enzyme, allowed for the buildup of glycolytic intermediates that could be used for anabolic processes that promote cell proliferation. Later studies considered a conditional knockout of PKM2-defining exon 10 in *PKM* and found that loss of the PKM2 isoform could accelerate the growth of autochthonous tumors (Israelsen et al., 2013); further reports showed that it could also disrupt anabolic metabolism (i.e., nucleotide synthesis) in certain settings (Lunt et al., 2015). At first glance, these findings appear to be incompatible. Nevertheless, the reconciling view was that lower pyruvate kinase activity (by either expression of PKM2 or loss of the enzyme altogether) in all cases was beneficial for anabolic metabolism and thus cell proliferation and tumor growth. Further complicating matters is that the model also proposed that this lower pyruvate kinase activity, in addition to promoting anabolic metabolism, would lead to an increase in glycolysis

and the Warburg Effect. However, a survey of the metabolic landscape related to the Warburg Effect appears to indicate that differences in pyruvate kinase activity may have little to no relationship with the accumulation of glycolytic intermediates, the overall glycolytic rate, or the Warburg Effect (Shestov et al., 2014).

A new study in this issue of *Cancer Cell* (Morita et al., 2018) sought to address some of this controversy by generating novel mouse models that express only a specific pyruvate kinase isoform. Rather than using a conditional deletion in exon 10, Morita and colleagues developed a clever targeting construct whereby the 3' end of the PKM1 or PKM2 cDNA, along with a polyA tail, is knocked in to the endogenous *PKM* at exon 8, the point in the gene prior to where the alternative splicing of its transcripts occurs. The authors establish that these mice not only retain tissue-specific expression of *PKM*, but also exhibit exclusive expression of PKM1 or PKM2 at levels commensurate with that of the endogenous gene. They then sought to evaluate the consequences of exclusive expression of PKM1 or PKM2 in cancer.

Strikingly, across numerous cancer models—including carcinogen-induced tumors, K-Ras^{G12D}-driven autochthonous lung adenocarcinomas, small-cell lung cancer and neuroendocrine tumor cell lines, fibroblast transformation assays, and allografts—expression of PKM1 promoted and PKM2 inhibited tumorigenesis relative to the wild-type control. Greater pyruvate kinase enzyme activity, a higher glycolytic rate, and increased anabolic metabolism were observed in the PKM1 system. Differences in other indirect mechanisms, such as autophagy and glutamine uptake, were also reported. Altogether, Morita and colleagues clearly demonstrate



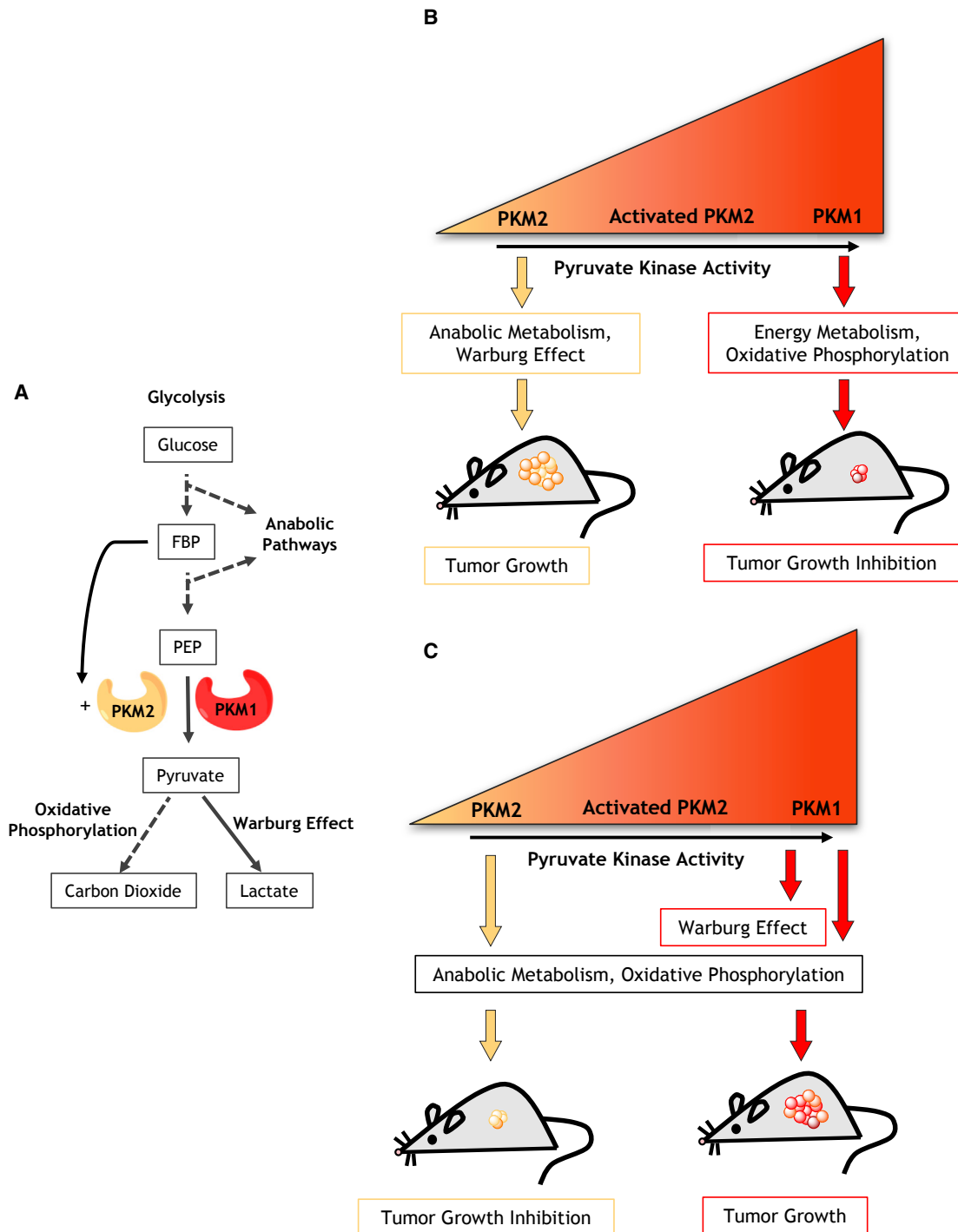


Figure 1. Pyruvate Kinase Isoform Expression in Cancer Metabolism

(A) Schematic of glucose metabolism in relation to PKM1 and PKM2. Solid arrows represent direct biochemical actions. Dotted arrows represent indirect biochemical actions. Orange and red denote lesser and greater enzyme activity, respectively.

(B) The previous model, in which PKM2 expression and low pyruvate kinase activity promotes anabolic metabolism, the Warburg Effect, tumor growth, and suppresses oxidative phosphorylation.

(C) In the new model, PKM1 expression and high pyruvate kinase activity favor the Warburg Effect, anabolic metabolism, and tumor growth, but this does not accompany decreased oxidative phosphorylation.

in their system that the functional genetics of pyruvate kinase isoform expression in cancer favors expression of PKM1, and thus increased activity of pyruvate kinase.

As in any landmark finding, there are many questions remaining. Is our idea of the role of PKM2 in cancer, thought to be oncogenic and known to be preferentially expressed in tumors, encountering a paradigm shift? Interestingly, nearly 30 years ago it became apparent that the hitherto cancer-promoting p53 gene—also highly expressed in many cancers—was indeed a tumor suppressor (Lane and Benchimol, 1990). Or, perhaps, does this study underscore the limitations of using mouse genetics to elucidate the role of a metabolic gene in a complex disease? Genetic experimentation is designed in general to produce binary outcomes, but metabolism, a product of the complex interaction between many genes and the nutritional environment, constitutes a quantitative continuum of phenotypes that do not resolve under these experimental setups. That is, pyruvate kinase expression is just one small part of the many factors that shape glycolysis, and an infinite number of mouse models may be needed to fully address the role of pyruvate kinase and glycolysis in cancer. Another interpretation of the apparent discrepancies this study raises is that metabolic pathway outcome does not uniquely map to any given metabolic

enzyme activity, such as pyruvate kinase. Thus, both enhanced and increased PKM activity can promote or suppress tumorigenesis, depending on the environment and status of all the other genes in the metabolic network (Liberti et al., 2017). Notably, similar questions also persist regarding the role of other metabolic processes such as AMPK activation, mTOR signaling, and autophagy that have each been reported to have both pro- and anti-cancer functions. Nevertheless, in light of this important study, it is anticipated that PKM1 may now become a focus of research interest, such as the development of agents that inhibit PKM1 activity, further defining its interaction with PKM2, or the investigation of possible new regulatory interactions and moonlighting functions specific to PKM1 that are currently very poorly understood.

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Targeting mRNA Decapping in AML

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In this issue of *Cancer Cell*, Yamauchi et al. identify a dependency of acute myeloid leukemia (AML) on DCPS, which catalyzes the final step of 3'-to-5' mRNA decay and is implicated in numerous aspects of RNA metabolism. DCPS is targetable with a clinical inhibitor, underscoring the translational importance of this discovery.

Acute myeloid leukemia (AML) is a heterogeneous malignancy characterized by accumulation of immature hemato-

poietic cells and impaired production of normal blood cells. Although a number of therapies have been recently FDA-

approved for AML, the outcome of the disease remains poor overall, and a great need for improved treatments remains. In

