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Perspective

Nonmetabolic functions of pyruvate kinase isoform M2 in controlling cell cycle progression and tumorigenesis

Zhimin Lu[1,2,3]

Abstract

Pyruvate kinase catalyzes the rate-limiting final step of glycolysis, generating adenosine triphosphate (ATP) and pyruvate. The M2 tumor-specific isoform of pyruvate kinase (PKM2) promotes glucose uptake and lactate production in the presence of oxygen, known as aerobic glycolysis or the Warburg effect. As recently reported in *Nature*, PKM2, besides its metabolic function, has a nonmetabolic function in the direct control of cell cycle progression by activating β-catenin and inducing expression of the β-catenin downstream gene *CCND1* (encoding for cyclin D1). This nonmetabolic function of PKM2 is essential for epidermal growth factor receptor (EGFR) activation-induced tumorigenesis.

Key words Pyruvate kinase, cell cycle, tumorigenesis

As noted by Warburg in the 1920s, tumor cells, unlike their normal adult counterparts, have elevated glucose uptake and lactate production in the presence of oxygen, with reduced mitochondrial oxidative phosphorylation for glucose metabolism. This effect, known as aerobic glycolysis or the Warburg effect, allows tumor cells to function like fetal cells and to use a large fraction of glucose metabolites for synthesizing macromolecules, such as amino acids, phospholipids, and nucleic acids, to support tumor cell growth [1]. Pyruvate kinase regulates the rate-limiting final step of glycolysis, which catalyzes the transfer of a phosphate group from phosphoenolpyruvate (PEP) to adenosine diphosphate (ADP), yielding one molecule of pyruvate and one molecule of adenosine triphosphate (ATP) [2,3]. Four pyruvate kinase isoforms exist in mammals: the L (PKL) and R (PKR) isoforms, which are expressed in liver and red blood cells; the M1 (PKM1) isoform, which is expressed in most adult tissues; and the M2 (PKM2) isoform, which is a splice variant of M1 expressed during embryonic development [4]. During tumorigenesis, the tissue-specific pyruvate kinase isoform is replaced by PKM2, which plays an essential role in aerobic glycolysis [5-7]. Although the enzymatic activity of PKM2 is half of that of PKM1, tumor formation in nude mouse xenografts is inhibited when PKM1 replaces PKM2 in cancer cells [8]. These findings indicate that PKM2 but not PKM1 plays a critical role in tumor growth.

The model to explain the unique role of PKM2 in contrast to PKM1 in tumorigenesis is that the slower rate of glycolysis catalyzed by PKM2, whose activity can be inhibited by oxidation of Cys358 and tyrosine phosphorylation, allows greater diversion of glycolytic intermediates into subsidiary pathways (such as the hexosamine, pentose phosphate, and amino acid biosynthetic pathways), thus supporting cellular biomass increase and generating sufficient reducing potential for the detoxification of reactive oxygen species (ROS) [1,9,10]. In addition, PKM2 binds to tyrosine-phosphorylated peptides, which results in the release of the allosteric activator fructose-1,6-bisphosphate, and this phosphotyrosine-binding form of PKM2 may promote diversion of glucose metabolites from energy production to anabolic processes [11]. Under hypoxic conditions, prolyl-hydroxylated PKM2 interacts with hypoxia-inducible factor 1 alpha (HIF1α) to induce glycolytic gene expression that promotes glucose metabolism in cancer cells [12]. These

Author's Affiliations: [1]Brain Tumor Center and Department of Neuro-Oncology, [2]Department of Molecular and Cellular Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA; [3]The University of Texas Graduate School of Biomedical Sciences at Houston, Houston, TX 77030, USA.

Corresponding Author: Zhimin Lu, [1]Brain Tumor Center and Department of Neuro-Oncology, [2]Department of Molecular and Cellular Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA; [3]The University of Texas Graduate School of Biomedical Sciences at Houston, Houston, TX 77030, USA. Email: zhiminlu@mdanderson.org.

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findings, however, may not provide the complete explanation of the specific roles of PKM2.

Besides the well-known role of PKM2 in glycolysis, Yang *et al.*[13] demonstrated that epidermal growth factor receptor (EGFR) activation, which occurs in many types of human tumors, results in the nuclear translocation of PKM2, but not of PKM1, in human glioblastoma multiforme (GBM) and breast and prostate cancers. Importantly, nuclear PKM2 directly regulates β-catenin transactivation, cell cycle progression, and tumorigenesis[13], illustrating a fundamental difference between PKM2 and PKM1 in cell proliferation and an important mechanism underlying PKM2-promoted tumor development.

β-catenin, functioning as a major component of the Wnt signaling and cell-cell adhesion, plays a central role in many aspects of cell function and development[14,15]. In contrast to activation of β-catenin by Wnt-dependent or activating mutations of Wnt pathway components, growth factor receptor activation transactivates β-catenin by distinct mechanisms[16]. In response to epidermal growth factor (EGF) treatment, β-catenin is transactivated not by the inhibition of glycogen synthase kinase-3β-dependent N-terminal phosphorylation and degradation of β-catenin, but instead by EGF-induced endocytosis of adheren protein complexes[17] and disruption of β-catenin interaction with E-cadherin and β-catenin, the latter of which is mediated by AKT-dependent phosphorylation of β-catenin at Ser552 and protein kinase CK2-dependent phosphorylation of β-catenin at Ser641, respectively[18,19].

The β-catenin released from the inhibitory adheren complex then translocates into the nucleus for induction of downstream gene transcription. However, how β-catenin is regulated in the nucleus in response to the EGF signaling is largely unknown. Yang *et al.*[13] discovered that EGFR activation results in phosphorylation of nuclear β-catenin Tyr333 by c-Src. Phosphorylated β-catenin Tyr333 serves as a binding motif for PKM2 Lys433. The interaction between PKM2 and β-catenin is required for this complex to interact with transcription factor 4 (TCF4) and to bind to the *CCND1* (encoding for cyclin D1) promoter, which is likely guided by the associated β-catenin. The binding of this complex to the promoter results in histone deacetylase 3 removal, acetylation of histone H3 in the promoter region, and subsequent cyclin D1 expression in a PKM2 kinase activity–dependent manner. In addition, the PKM2/β-catenin complex is required for EGF-induced, but not Wnt-induced, and β-catenin transactivation-dependent expression of downstream target genes such as *c-myc* and Dickkopf 1 (*DKK1*), cell cycle progression, cell proliferation, and tumor cell migration and invasion[13]. This PKM2-regulated β-catenin transactivation, which is distinct from Wnt-dependent canonical regulation of β-catenin, broadens the understanding of the role and regulation of β-catenin in EGFR activation-induced cellular activities. It also provides a mechanism to explain the clinical observation that β-catenin transactivation, independent of Wnt or mutations of Wnt signaling components, has been detected in many types of cancer[16].

The importance of PKM2-regulated β-catenin transactivation is highlighted by its role in tumorigenesis and potential clinical impact. Expression of EGFRvIII, which lacks 267 amino acids from its extracellular domain and is commonly found in human GBM and in breast and lung cancers, results in rapid brain tumor xenograft growth in mice, which was completely blocked by depletion of PKM2 or β-catenin[13]. Reconstituted expression of β-catenin Y333F, or a PKM2 K433E mutant that retains its catalytic activity for glycolysis failed to rescue the effect of the depletion of β-catenin or PKM2 on tumor growth, further supporting an essential nonmetabolic role of PKM2 in tumorigenesis.

Levels of β-catenin Tyr333 phosphorylation and nuclear PKM2 have also been correlated with grades of glioma malignancy and prognosis and can be potential biomarkers in clinical applications[13]. This impact is further broadened by the finding that treatment with a c-Src inhibitor, which inhibits β-catenin Tyr333 phosphorylation, blocked EGFRvIII-induced tumor growth. Given that EGFR-based therapy is not very efficient due to drug resistance, this discovery can be of special importance because levels of β-catenin Tyr333 phosphorylation can potentially serve as a guideline for personalized cancer therapy in treating glioma and other tumors with Src inhibitors.

In summary, the demonstration of a novel nonmetabolic role of PKM2 in directly promoting cell proliferation by transactivation of β-catenin provides an important insight in understanding the roles of tumor-specific PKM2 in human cancer development. The coordinated control of metabolism and cell cycle progression by metabolic and nonmetabolic functions of PKM2 is essential for tumorigenesis.

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