The Multifunctionality of M2-type Pyruvate Kinase in Tumor Cells: Postprint

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Abstract

Pyruvate kinase is a key rate-limiting enzyme in glucose metabolism. It exists primarily in four isozyme forms: L-type, R-type, M1-type, and M2-type. M2-type pyruvate kinase is predominantly expressed in tumor cells. Glucose metabolism in tumor cells is mainly accomplished through aerobic glycolysis, a process in which M2-type pyruvate kinase serves as a key rate-limiting enzyme and also plays a role in regulating signal transduction during tumor cell proliferation. M2-type pyruvate kinase provides the energy and material basis for tumor cell proliferation and can serve as a biomarker for early tumor detection. A thorough understanding of the role and mechanism of M2-type pyruvate kinase in tumors can provide novel insights for tumor-targeted therapy.

Full Text

Preamble

Versatility of Pyruvate Kinase M2 in Tumor Cells

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Abstract

Pyruvate kinase is a critical rate-limiting enzyme in glucose metabolism that exists in four main isozyme forms: L-type, R-type, M1-type, and M2-type.

Pyruvate kinase M2 (PKM2) is predominantly expressed in tumor cells. Tumor cells primarily metabolize glucose through aerobic glycolysis, a process in which PKM2 serves as the key rate-limiting enzyme while also regulating signal transduction during tumor cell proliferation. PKM2 provides the energetic and material basis for tumor cell proliferation and can serve as a biomarker for early tumor detection. A deeper understanding of PKM2' s role and mechanisms in tumors may offer new insights for targeted cancer therapy.

Keywords: M2-type pyruvate kinase; tumor; glycolysis; non-glycolysis

A critical factor for cell survival is obtaining adequate energy supply. Tumor cells utilize glycolysis to produce energy for survival under hypoxic conditions, a phenomenon known as the Warburg effect [1]. Studies have confirmed that PKM2 is a key regulatory enzyme in glycolysis, providing the material basis for cell proliferation by supplying nucleic acids, amino acids, lipids, and other building blocks [2]. Beyond its glycolytic function, the non-glycolytic functions of PKM2 have attracted widespread attention. This review describes the functions of PKM2 in tumor cells and explores its potential therapeutic applications.

1. Four Isoforms of Pyruvate Kinase

Pyruvate kinase is a glycolytic enzyme that catalyzes the conversion of phosphoenolpyruvate and ADP to pyruvate and ATP. Four isoforms exist (PKL, PKR, PKM1, and PKM2). The L-type and R-type are encoded by the PKLR gene and exhibit tissue-specific expression patterns: L-type is primarily expressed in liver, kidney, and intestinal tissues, while R-type is mainly expressed in red blood cells [3]. PKM1 and PKM2 are encoded by the PKM gene as alternative splicing products. PKM1, which contains exon 9, is predominantly expressed in differentiated adult tissues such as brain and muscle [4,5]. PKM2, which contains exon 10, is mainly expressed in embryonic cells, adult stem cells, and tumor cells [6,7].

2. PKM2 Expression in Tumor Cells

PKM2 represents the predominant form in tumor cells, in addition to its presence in embryonic and adult stem cells [8,9]. PKM2 expression plays a crucial role in tumor growth [10,11]. Recent studies have identified mTOR as a regulator of PKM2 expression; mTOR activation leads to increased PKM2 expression, which in turn transcriptionally activates hypoxia-inducible factor (HIF-1), thereby promoting tumorigenesis. Consequently, PKM2 serves as a biomarker for early tumor detection.

3. Glycolytic Function of PKM2

In the Warburg effect, PKM2 exists as a low-activity enzyme, creating favorable conditions for tumor development. First, the glycolytic pathway produces ATP more rapidly than oxidative phosphorylation while enabling faster synthesis of

carbon-containing organic compounds, thereby providing abundant material resources for tumor growth [12,13]. Research has confirmed that glycolysis can generate ATP rapidly through high glucose consumption, though the amount of ATP produced is relatively low [14]. Second, low-activity PKM2 promotes the accumulation of glycolytic intermediates such as nucleic acids, providing the material foundation for tumor development [15,16]. In summary, low-activity PKM2 enhances glycolysis, supplying tumor cell proliferation with various resources including energy and biosynthetic precursors.

PKM2 exists in three activity states: inactive monomers, low-activity dimers, and high-activity tetramers, whereas PKM1 primarily exists as high-activity tetramers [17]. In tumor cells, PKM2 mainly adopts the low-activity dimeric form [18], while normal proliferating cells predominantly contain the high-activity tetrameric form [8]. Studies have reported that cells expressing PKM1 exhibit higher pyruvate kinase activity than those expressing PKM2, consume more oxygen, produce less lactate, and are more sensitive to oligomycin, an inhibitor of mitochondrial ATP production [19,20]. In tumor cells, serine and tyrosine residues on PKM2 undergo phosphorylation. Phosphotyrosine on PKM2 promotes the release of fructose-1,6-bisphosphate from the PKM2 binding pocket, causing tetramers to dissociate into inactive dimers and shifting glycolysis toward biosynthesis [21]. This enhances aerobic glycolysis capacity and accelerates tumor progression. The E7 protein, an oncoprotein from human papillomavirus type 16, can exacerbate PKM2 dimerization and accelerate tumor development when bound to PKM2.

Multiple factors can regulate the interconversion between PKM2 dimers and tetramers [22-24]. For example, the glycolytic intermediate fructose-1,6-bisphosphate can allosterically regulate PKM2 by converting dimeric PKM2 into tetramers. However, phosphotyrosine on PKM2 promotes the release of fructose-1,6-bisphosphate from the PKM2 binding pocket, causing tetramers to dissociate into inactive dimers and shifting glycolysis toward biosynthesis. Tetrameric PKM2 is associated with high ATP:ADP and GTP:GDP ratios, whereas dimeric PKM2 is associated with the opposite condition. Serine, derived from the glycolytic intermediate 3-phosphoglycerate, also serves as a regulator of PKM2 [25]. Additionally, oncogenic proteins HPV-16 E7 and active pp60V-Src can convert tetrameric PKM2 into dimeric PKM2 upon binding [8]. Recent studies have found that hyperoxic environments can cause dissociation of PKM2 tetramers, thereby reducing PKM2 activity [26,27]. Furthermore, acetylation of PKM2 lysine residues inhibits PKM2 enzymatic activity, leading to degradation through chaperone-mediated autophagy [28].

4. Non-glycolytic Function of PKM2

PKM2 can interact with numerous molecules [29-31], some of which affect its glycolytic function and directly regulate the Warburg effect. Extensive research has reported on PKM2' s non-glycolytic functions, particularly its role in transcription. Studies have shown that PKM2 can directly interact with HIF-1 to

promote transcriptional activation of HIF-1 target genes [32]. Numerous reports have also documented PKM2 nuclear translocation. Interleukin-3 and epidermal growth factor receptor activation can induce PKM2 nuclear translocation, thereby activating gene transcription and cell proliferation [33]. When cells receive growth factor signals, PKM2 activity is inhibited, which enhances the glycolytic pathway and leads to accumulation of glucose metabolites, providing energy and material basis for cell proliferation. A research team at the University of Texas also revealed that PKM2 promotes cell proliferation and tumor formation through a non-metabolic mechanism [34]. Their study demonstrated that PKM2 is essential for epidermal growth factor receptor signaling, enhances -catenin activity, and consequently induces gene expression, cell growth, and tumor formation. The study confirmed that in human cancer cells, epidermal growth factor receptor signaling activation induces PKM2 translocation into the nucleus, where K433 of PKM2 binds to the c-Src-phosphorylated Y333 site of -catenin, thereby regulating Cyclin D1 expression and leading to accelerated cell proliferation and tumor formation.

Nuclear PKM2 primarily exists as dimers, whereas cytoplasmic PKM2 exists as both dimers and tetramers [35]. Additionally, nuclear PKM2 functions as a protein kinase that can phosphorylate Stat3 to activate transcription of tumor-associated genes such as Mek5 [36]. Collectively, these studies suggest that PKM2 plays dual roles in promoting tumor development: first, dimeric PKM2 in the cytoplasm functions as a pyruvate kinase, where low PKM2 activity sustains glycolysis and promotes generation of glycolytic intermediates, providing the material basis for tumor cell proliferation; second, dimeric PKM2 in the cytoplasm translocates into the nucleus and functions as a protein kinase that can phosphorylate specific nuclear proteins to promote gene transcription, thereby driving tumor cell proliferation. Recent studies have found that PKM2 exhibits different functions under various environmental conditions and is associated with immune responses, genomic instability, angiogenesis, and pathogenesis [37]. Whether these disease processes are related to PKM2' s glycolytic or non-glycolytic functions requires further investigation.

5. Prospects of PKM2 as a Therapeutic Target

Given its numerous important roles in tumor cell metabolism and signal transduction, PKM2 is considered an ideal therapeutic target for cancer. RNA interference and peptide aptamers can ablate PKM2, producing anti-tumor effects such as inhibiting tumor growth, inducing apoptosis, and increasing chemosensitivity [38]. Small molecule inhibitors of PKM2 can suppress glycolysis and cause cell death. However, targeting PKM2 remains challenging for two main reasons: first, PKM2 is expressed not only in tumor tissues but also in normal proliferating tissues; second, gene silencing of PKM2 via small interfering RNA cannot completely inhibit tumor cell proliferation. Therefore, compounds that activate PKM2 catalytic activity may represent a therapeutic approach [39]. PKM2 exists in a high-activity form in normal tissues but a low-activity form in tumor

tissues. Activators can inhibit tumor cell glycolysis and proliferation by inducing tetramer formation in PKM2, similar to fructose-1,6-bisphosphate. Since nuclear PKM2 exists as dimers, PKM2 activators can prevent PKM2 translocation from the cytoplasm to the nucleus, thereby inhibiting nuclear PKM2 function. In other words, PKM2 activators may suppress both glycolytic and non-glycolytic functions of PKM2. Inhibitors of PKM2 glycolytic function can suppress tumor cell glycolysis, leading to increased ROS production and reduced supply of energy materials required for rapid tumor cell growth. Inhibitors of PKM2 non-glycolytic function can suppress tumor-associated genes such as Mek5, c-Myc, and various HIF-1 target genes. The therapeutic rationale for these approaches requires further investigation.

6. Outlook

The glycolytic and non-glycolytic functions of PKM2 provide the necessary conditions for tumor cell growth and survival. PKM2 expression occurs during early tumor formation and can be detected in the blood and feces of cancer patients [19]. Furthermore, studies have shown that PKM2 levels correlate with tumor size and stage.

The tumor microenvironment can induce metabolic heterogeneity in tumor tissues, which may result from differential supply of energy materials and oxygen to individual tumor cells due to their varying distances from blood vessels. PKM2 creates conditions for the malignant phenotype of tumor cells through both glycolytic and non-glycolytic functions, suggesting that PKM2 may become an effective therapeutic target. However, PKM2 is multifaceted, with numerous and complex intracellular functions, and the effects of targeting PKM2 in normal cells are difficult to evaluate. Therefore, further research is needed before PKM2 activators and inhibitors can be used as therapeutic interventions.

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