Review

Dual roles of PKM2 in cancer metabolism

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Cancer cells have distinct metabolism that highly depends on glycolysis instead of mitochondrial oxidative phosphorylation alone, known as aerobic glycolysis. Pyruvate kinase (PK), which catalyzes the final step of glycolysis, has emerged as a potential regulator of this metabolic phenotype. Expression of PK type M2 (PKM2) is increased and facilitates lactate production in cancer cells, which determines whether the glucose carbons are degraded to pyruvate and lactate or are channeled into synthetic processes. Modulation of PKM2 catalytic activity also regulates the synthesis of DNA and lipids that are required for cell proliferation. However, the mechanisms by which PKM2 coordinates high-energy requirements with high anabolic activities to support cancer cell proliferation are still not completely understood. This review summarizes the biological characteristics of PKM2 and discusses the dual role in cancer metabolism as well as the potential therapeutic applications. Given its pleiotropic effects on cancer biology, PKM2 represents an attractive target for cancer therapy.

Keywords   aerobic glycolysis; pyruvate kinase isoenzyme M2; cancer metabolism; cell proliferation

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Introduction

Hanahan and Weinberg [1] have proposed that different types of cancers share 10 common basic characteristics. Among these, reprogramming of the metabolic pathways, especially reprogramming of energy metabolism, is an important feature, representing the physiological changes that occur in cancer cells. In the 1920s, Otto Heinrich Warburg first pointed out that metabolic disorders, especially disorders of cellular respiration, are a common feature of cancer [2]. Under aerobic conditions, through glycolysis, the tricarboxylic acid (TCA) cycle, and oxidative phosphorylation, in terminally differentiated cells, one glucose molecule is fully oxidized to produce CO_2, and H_2O, and 36 ATP molecules. Only under hypoxic conditions can cells generate two lactic acid and two ATP molecules through anaerobic respiration. However, Warburg found that, unlike normal cells, cancer cells can generate energy through rapid glycolysis even in the presence of ample oxygen. This is accompanied by the production of large amounts of lactic acid molecules, and the oxidative phosphorylation process is inhibited [3]. This metabolic phenomenon is often called the Warburg effect or aerobic glycolysis. Initially, Warburg [4] thought that aerobic glycolysis was caused by mitochondrial damage in the cancer cells. However, other studies have shown that, under certain circumstances, cancer cells can switch back to mitochondrial respiration and that cancer cells with normal mitochondrial function grow faster than those without [5]. In this way, aerobic glycolysis in cancer cells is not likely to be due to irreversible damage to the mitochondria.

In 1910, Peyton Rous discovered the infectious nature of sarcoma extract. Many years later, a series of tumor viruses, oncogenes, and proto-oncogenes were discovered. Sarcoma virus transforming factor pp60[v-src] was discovered in 1977 and later shown to be a type of protein-tyrosine kinase [6,7]. Many studies have suggested that oncogenes and proto-oncogenes have direct and indirect relationships with metabolic reprogramming. During the early stages of tumorigenesis, the activation of oncogenes and changes in certain transcription factors up-regulate the majority of genes involved in glycolysis and specific isoenzymes are expressed, causing extensive changes in the tumor metabolomics. The M2 isoform of pyruvate kinase (PKM2 or M2-PK) has been shown to be a target of pp60[v-src], and it is continuously expressed during cancer progression [8]. Over the past three decades, many researchers have focused on metabolomics and signal transduction. Studies have demonstrated that the expression and activity of PKM2 are regulated by many oncogenes. For this reason, PKM2 is

considered a link between oncogenes and metabolism and thought to play a key role in the reprogramming of cancer metabolism and the proliferation of cancer cells. This paper reviews the dual role of PKM2, specifically its roles as a switch and speed regulator, in the proliferation of cancer cells, starting with the biological characteristics of PKM2.

The Biological Characteristics of PKM2

Four isozymes of pyruvate kinase

Pyruvate kinase (ATP: pyruvate 2-O-phosphotransferase, EC2.7.1.40, abbreviated as PK) catalyzes the dephosphory-lation of phoshphoenolpyuvate (PEP) to produce pyruvate and ATP. In this process, no oxygen is needed. This is particularly important in the growth of tumor cells in hypoxic environments.

There are four isozymes of PK in mammals, and these vary largely in tissue specificity, kinetic characteristics, and regulatory mechanism. PKL and PKR are products of the same *PKL* gene, transcribed with different promoters. PKM1 and PKM2, however, are produced by alternative splicing of the *PKM* gene. *PKM* contains 12 exons, and the alternative splicing of exons 9 and 10 produces two isozymes [9,10]. In individual development, during the stage of embryogenesis, cells mainly express PKM2. As embryogenesis progresses, the expression of PKM2 is gradually replaced by that of tissue-specific PKM1/L/R. During tumorigenesis, tissue-specific PKM1/L/R expression gradually diminishes and is replaced by PKM2 expression [5,8].

Regulation of PKM2 expression

PKM2 expression is regulated by multiple signal pathways at different levels. Insulin can induce the expression of the *PKM* gene through activating the PI3K/MAPK signal pathway. During *PKM* expression, two transcription factors, SP1 (Specificity Protein 1) and SP2 (Specificity Protein 2), play key roles. Glucose can induce the phosphorylation of SP1, enhancing its ability to bind to DNA and so increasing the expression of PKM2. Ras can also substantially decrease the activity of promoters regulated by SP1 [11,12]. Hypoxia-inducing factor 1 (HIF-1) is an important transcription factor, and it is often highly expressed in hypoxic tissues and cancer cells. It can up-regulate many types of metabolic enzymes, such as glucose transporter 1, hexokinase, and lactate dehydrogenase (LDH). High-level expression of these enzymes is a key to cancer metabolism. Recently, Luo *et al.* demonstrated that the first intron in PKM2 contains a hypoxia response element, and it can be up-regulated by HIF-1. Conversely, as a coactivator, PKM2 can also increase the transcriptional activity of HIF-1. Therefore, PKM2 and HIF-1 can form a positive feedback loop and regulate the expression of the target genes of

HIF-1, thus promoting metabolic reprogramming and angiogenesis [13]. Another recent study also suggested that the RTK/PI3K/AKT/mTOR signal pathway can up-regulate the expression of PKM2 via HIF and c-Myc [14].

After transcription, pre-mRNA are spliced alternatively by three heterogeneous nuclear ribonucleoproteins (hnRNPs) hnRNPA1/A2/I which up-regulated due to the oncogenes c-Myc amplification. HnRNPA1/A2/I could bind to exon 9 of the pre-mRNA so that it could be spliced off to form mature PKM2-mRNA [15,16]. MicroRNA is a type of non-coding, small RNA molecule. It plays an important role in the regulation of gene expression. Studies have shown that over-expression of PKM2 in squamous cell carcinoma of tongue is related to the down-regulation of microRNA133a and microRNA133b [17].

In addition to regulation at the DNA and RNA levels, PKM2 is also modified post-transcriptionally by phosphorylation, hydroxylation, acetylation, sumoylation, and ubiquitination. These modifications play important roles in regulation of the activity and function of PKM2. *In vivo* PKM2 can transform between monomers, dimers, and tetramers. The transformation between dimers and tetramers is particularly important to the reprogramming of cancer metabolism [5,8]. In fine, PKM2 is regulated by transcription factors and oncoproteins at different levels as it plays its roles in cancer metabolism.

The structural features of PKM2

As shown in **Fig. 1** , PKM2 is composed of four domains. A-domain participates in the formation of dimers. C-domain mediates the interactions between dimers that allow them to form tetramers. The active sites of PK are located in domains A_2 and C. Out of the 56 amino acids in exon 10, 44 are located in C-domain. It is indicated that C-domain is critical to the formation of PKM2 quaternary structure [18]. The most notable difference between PKM2 and the other three isozymes is that *in vivo* PKM2 exists in two forms, dimers and tetramers. In normal cells, PKM2 mainly exists in the tetramer form, but in cancer cells it mainly exists as dimers [5,8,19].

The catalytic properties of PKM2

Kinetic studies of PKM2 performed by Mazurek *et al.* [8] showed that, in MCF-7, for tetrameric PKM2, the K_m of the substrate PEP was 0.03 mM, but for dimeric PKM2, it was as high as 0.46 mM. This suggests that, at physiological concentrations (15.0–19.0 μM, data from http://www.hmdb.ca/metabolites/HMDB00263), tetramer activity is high and dimers are almost inactive. In addition to using ADP as a substrate, PKM2 can also catalyze the phosphorylation of GDP at the substrate level. In this process, its activity is relatively low, but there is no significant difference between the activity of dimers and that of the tetramers [8]. One other factor is
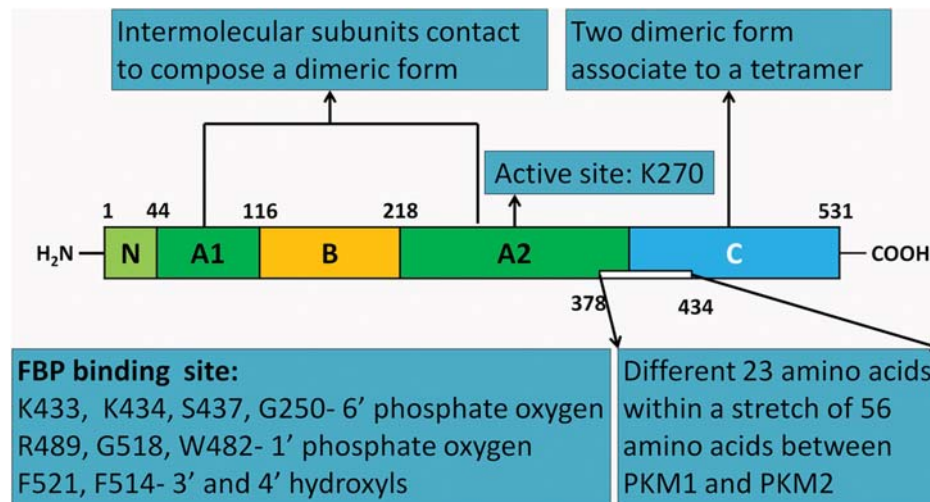
Figure 1 Scheme of PKM2 structural features.

also crucial to aerobic glycolysis: tetrameric PKM2 and other glycolytic enzymes, including phosphoglycerate mutase, glyceraldehyde-3-phosphate dehydrogenase, adenylate kinase, phosphoglycerate dehydrogenase, glycerol-3-phosphate dehydrogenase, and LDH, can form the so-called glycolytic enzyme complex [20]. Studies have shown that tetrameric PKM2 can also bind with some enzymes in the glycolytic sub-pathways and the protein kinase cascade. Therefore, these changes in PKM2 contribute glucose to lactic acid and ATP in a highly effective manner [5,8,20].

The regulation of PKM2 by metabolic intermediates

PK is a key enzyme in the last step of glycolysis, and its activity is regulated by many metabolic intermediates. Among these, intermediate fructose-1,6-bisphosphate (FBP) is an important allosteric activator of PKM2. The high concentration of the nearly inactive dimeric PKM2 causes FBP to accumulate gradually. When dimers reach a certain concentration, tetramerization of PKM2 is induced, the activity of PKM2 is enhanced and the rate of glycolysis is also increased. This in turn causes the amount of FBP to decrease continuously and eventually leads to dimerization of PKM2 [18]. This type of interaction between PKM2 and FBP maintains equilibrium between the two. Together, they regulate the flow of glycolytic intermediates.

In addition to classical glycolytic intermediates, many other intermediates in glycolytic sub-pathway have been found to regulate the activities of PKM2. Another important activator is serine (Ser), which is produced through the transamination of glycerate-3-phosphate (G3P). Latest studies show Ser is a natural ligand and allosteric activator of PKM2. Ser can bind to and activate PKM2, which result in *de novo* serine synthesis to support cell proliferation [21,22]. SAICAR (succinylaminoimidazolecarboxamide

ribose-5′-phosphate, an intermediate of the *de novo* purine nucleotide synthesis pathway) can specifically stimulate PKM2 upon glucose starvation resulting to alter the cellular energy level, glucose uptake, and lactate production [23]. What's more, Cys, Met, Phe, Val, leu, Ile, Pro, and both saturated and monounsaturated fatty acids can also inhibit the activity of PKM2. Thyroid hormone 3 (3′5′-iodo-L-thyronine, T3) can bind to PKM2 monomers and inhibit the tetramerization of PKM2 [5,8,18].

The phosphorylation of PKM2 and its effect on the activity of PKM2

Phosphorylation/de-phosphorylation is an important mechanism to regulate the activity of enzymes in organisms. Studies have shown that PKM2 can be phosphorylated by many kinases. Tyrosine kinase has been found to be highly expressed in many types of cancers, playing an important role in the regulation of cancer cell growth [24]. In 2008, Christofk *et al*. [25] found PKM2 to be a phosphotyrosine-binding protein. One year later, Hitosugi *et al*. found that fibroblast growth factor receptor 1 could directly phosphorylate multiple Tyr residues in PKM2 by proteomics studies. Phosphorylated PKM2 at Y105 disturbs the binding between PKM2 and its allosteric agent FBP, inhibiting the formation of tetramers and ultimately promoting aerobic glycolysis and tumor growth. Hitosugi *et al*. [26] also reported that, in different cancer cell lines, BCL-ABL, JAK2, FLT3, and ETV6-NTRK3 can also cause the tyrosine phosphorylation of PKM2. In addition, viral proteins pp[60v-src] and HPV-16 E7 can catalyze the tyrosine phosphorylation of PKM2, inhibiting the tetramerization of PKM2 and promoting cancer cell proliferation [27,28]. Aside from tyrosine phosphorylation, A-Raf and PCKδ can phosphorylate the Ser sites of PKM2. A-Raf can inhibit the

tetramerization of PKM2, and PCKδ may be involved in the stability of PKM2 [5,29].

Other post-transcriptional modifications and functions
Many other post-transcriptional modifications also regulate the activity and function of PKM2 (**Table 1**). In 2007, Hoshino *et al*. reported that the C terminal of PKM2 contained inducible nuclear translocation signal. PKM2 was found to enter the nuclei when induced by interleukin-3, and this process was dependent on the activity of JAK2 [30]. In 2009, Spoden *et al*. [31] found that PKM2 could interact with SUMO-E3 ligase, and this interaction causes PKM2 to enter the nuclei. PKM2's interaction with HIF-1 depends on the hydroxylation modification of PKM2 by prlyl hydroxylase 3 [13]. Lv *et al*. [32] recently reported that acetyltransferase P300/CBP-associated factor (PCAF) could catalyze the acetylation of PKM2 at K62 and K305, and acetylation of PKM2 at K305 can promote PKM2 degradation and activating chaperone-mediated autophagocytosis. Garcia-Gonzalo *et al*. [33] found that PKM2 could interact with E3 ubiquitin ligase HERC1, but no ubiquitination of PKM2 was observed. The effect of this interaction merits further investigation.

In addition to changing the activity of PKM2 by modifying PKM2, some proteins interact with PKM2 directly and so change its activity. Shimada *et al*. [34] found that, in cytoplasm, promyelocytic leukemia protein could bind to PKM2 and inhibit the activity of tetrameric PKM2. Phosphorylated PKM2 can act as kinase to catalyze the phosphorylation of prothymosin, thus activating prothymosin [35]. As shown in **Table 1**, PKM2 can also interact with many proteins and plays important roles in the immune escape of cancer cells and pathogen infection [5,8].

The Roles of PKM2 in Cancer Metabolism

PKM2 of low activity provides cancer cells an ample budget of metabolic intermediates
Before cell division, all cell contents must be duplicated, and each duplication requires a large amount of nucleotides, amino acids, and lipids for the synthesis of biological macromolecules. In addition to generating energy, glucose must also provide a large amount of precursor substances, and the production of energy and precursor substances must occur at an appropriate ratio in order to maintain rapid cancer cell growth [36]. For example, palmitic acid, one of the main components of biological membranes, is a suitable example: to synthesize 1 palmitic acid molecule, 8 acetyl coenzyme A molecules, 7 ATP molecules, and 14 NADPH molecules are required. Full oxidation of 1 glucose molecule can provide enough energy for the synthesis of 5 palmitic acid molecules, but an additional 7 glucose molecules are needed to provide the 14 NADPH molecules through

the pentose phosphate pathway, and 3 glucose molecules are needed to provide the coenzyme A molecules that serve as the carbon skeleton [37]. Similarly, in the synthesis of amino acids, nucleotides, and other small-molecule precursors, the need for carbon skeleton and reducing power is far higher than the need for ATP. From this perspective, it is reasonable that aerobic glycolysis occurs in cancer cells and other cells that proliferate quickly.

Cancer cells express PKM2, which mainly exist in the form of the almost inactive dimers. This seems inconsistent with the fact that, in cancer cells, fast glycolysis produces a large amount of lactic acid. However, careful analysis reveals that it is in fact the low-activity PKM2 that allows smooth aerobic glycolysis and promotes proliferation. It is the key enzyme in the last step of glycolysis and helps cancer cells to accumulate metabolic intermediates upstream of PEP, thus providing an ample supply of metabolic intermediates for the synthesis of precursor substances [5]. For example, the glycolytic intermediate G3P is the precursor of Ser, Gly, and Cys. Ser is also a donor of single-carbon units. After its methyl group is transferred to tetrahydrofolate, different types of active single-carbon units that play important roles in the *de novo* synthesis of purine are formed. Ser is also involved in the synthesis of phospholipids and sphingolipids. The majority of precursors of other nucleotides, lipids, and amino acids, such as 5-phosphoribosyl pyrophosphate, glycerol, and alanine, are all derived from glycolysis. The generation of these precursor substances requires fast glycolysis and the accumulation of a large amount of intermediate products. In the process of synthesis of small molecules, the intermediate metabolites, for example, Ser and SAICAR, can regulate the activity of PKM2 [21,23]. Low-activity PKM2 can prevent the outflow of the products of glycolysis, allowing intermediates to accumulate. Tetrameric PKM2 can form complexes with other enzymes, and after PEP is transformed into pyruvate, lactic acid can be formed quickly, meeting the cell's need for energy [20]. Low-activity PKM2 can keep ATP and GTP levels low, thus continuously activating phosphofructokinase-1, so that the rate of glycolysis remains high.

PKM2 as a switch for cancer cell proliferation and a regulator for the rate of proliferation
As mentioned, PKM2 is expressed specifically in cancer cells. Unlike PKM1/L/R, it can transform between tetramers and dimers. Dimerization of some PKM2s prevents glucose from entering glycolysis from and converting to pyruvate. Metabolic intermediates upstream of PEP are accumulated, and precursors of macromolecules, such as nucleotides, amino acids, and lipids, are then synthesized. In this way a material basis for cell proliferation is obtained. PKM2 acts like a switch. Its expression in cancer cells opens the door to cell proliferation. Tetrameric PKM2,

Table 1 Proteins interacting with PKM2

Interacting protein	Description	Interaction with PKM2	Effects
A-Raf	Isonenzyme of the Raf-kinase component of the protein kinase cascade	Binds to PKM2, phosphorylates PKM2 in serine	Tetramerization or dimerization of PKM2 depending on metabolism
BCR-ABL	Fusion of breakpoint cluster region and ABL1	Phosphorylates PKM2 in tyrosine	Disruption of the formation of the tetrameric form of PKM2
ETV6-NTRK3	Fusion of Est variant 6 and neurotrophic tyrosine kinase receptor	Phosphorylates PKM2 in tyrosine	Disruption of the formation of the tetrameric form of PKM2
FGFR-1	Fibroblast growth factor receptor	Binds to PKM2, phosphorylates PKM2 in tyrosine	Disruption of the formation of the tetrameric form of PKM2
FLT3	Fms-related tyrosine kinase, internal tandem duplication (ITD) mutant	Phosphorylates PKM2 in tyrosine	Disruption of the formation of the tetrameric form of PKM2
HCV NS5B	RNA polymerase of hepatitis C virus	Binds to PKM2	Indications for a role of PKM2 in HCV RNA synthesis
HERC-1	Acronyms: oncH, p532, consists of one HECT and two RLD domains	Binds to PKM2 aa 406–531	Hypothesis: GTP producer for guanine nucleotide exchange factor RLD1
HIF-1α	Hypoxia-inducible factor-1α	Binds to PKM2	Augmentation of the trans-activating activity of HIF-1α
HPV-16 E7	E7 oncoprotein of the human papilloma virus type 16	Binds to PKM2	Dimerization and inhibition of PKM2
HSP70	Heat-shock protein 70	Binds to acetylated PKM2	Mediation of lysosome translacation and degradation of PKM2
JAK2	Janus kinase 2	Phosphorylates PKM2 in tyrosine	Disruption of the formation of the tetrameric form of PKM2
Oct 4	Transcription factor involved in maintaining the pluripotent state of embryonic stem cells	Binds to PKM2 aa 307–531	Augmentation of the trans-activating activity of Oct 4
Opa	Outer membrane proteins involved in gonococcal adhesion to and invasion of human epithelial cells	Binds to PKM2 aa 367–531	Hypothesis: creation of a microenvironment of high pyruvate concentration
PANK-4	Pantothenate kinase 4	Binds to PKM2	Hypothesis: regulation of PKM2 activity
PCAF	P300/CBP-associated factor	Acetylates PKM2	Reduction of the activity of PKM2
PHD3	Prolyl hydroxylase domain-containing protein 3	Hydroxylates PKM2	Mediation of PKM2 interacting with HIF-1α
PIAS3	SUMO E3-ligase	Binds to PKM2 aa 1–348	Sumoylation of PKM2 and nuclear translocation of PKM2
PKCδ	Isoenzyme of the protein kinase C	Phosphorylates PKM2 in serine	Hypothesis: regulation of stability or degradation of PKM2
PML	Promyelocytic leukemia tumor suppressor protein	Binds to PKM2	Reduction of the activity of the tetrameric form
pp60v-src	Transforming principle of the Rous Sarcoma virus	Phosphorylates PKM2 in tyrosine	Dimerization and inhibition of PKM2
SOCS3	Suppressor of cytokine signaling 3	Binds to PKM2	Reduction of ATP production and influence of dendritic cell immune response
T3	Thyroid hormone 3,3,5-triiodo-ʟ-thyronine	Binds monomeric PKM2	Prevention of association of PKM2 to the tetrameric form
TEM8	Human tumor endothelial factor 8	Binds to PKM2 aa 379–385	Hypothesis: stimulation of angiogenesis by binding of Tumor PKM2 released from tumors

however, forms complexes with enzymes involved in glycolysis, so that glucose can be transformed into lactic acid and ATP in an efficient fashion. NADH is oxidized so that glycolysis can continue in tumor tissues in under hypoxic or anoxic conditions. The transformation of PKM2 from dimer to tetramer changes its activity and can affect the ratio of energy to precursor substances and regulate the speed of cell proliferation.

However, not all cancer cells consume large amounts of glucose. In fact, some cancer cells instead use large amounts of glutamine and can proliferate at very low glucose concentrations. These cells take up large amounts of glutamine, which after two deaminations forms α-ketoglutarate and enters the TCA cycle. It is ultimately is transformed into aspartate, pyruvate, CO_2, lactic acid, alanine, and citric acid. Citric acid can be degraded into acetyl-CoA in the cytoplasm [5,37,38]. Glutamine metabolism can be considered an incomplete form of the TCA cycle. Glutaminolysis can combine with serine synthesis to balance the utility of glucose and glutamine in some cancer cells [39,40]. It both provides precursors to the synthesis of amino acids and fatty acids for cell proliferation and generates ATP through oxidative phosphorylation. In addition, degradation of malic acid produces not only pyruvate but also the reducing power NADPH. In this type of cell, PKM2 also plays a key role. Dimeric PKM2 can cause limited amounts of glucose to be transformed into glycolytic intermediates for biological synthesis; on the other hand, the coupling between the G3P transformation into Ser and the glutamate deamination links the two metabolic pathways together [39,40]. In this process, PKM2 also regulates the ratio of energy to precursor substances.

PKM2 as a multifunctional signaling molecular in the nucleus

As mentioned above, PKM2 can be relocated in the nucleus, which indicates that there may be an important role in the nucleus. In the cytoplasm, tetramer is its active form. However, within the nucleus, it becomes a protein kinase using PEP as a phosphate donor, and the dimer is its active form [41]. Gao *et al*. [41] have shown that PKM2 is able to phosphorylate stat3 at Y705 and promote transcription of MEK5. Again, Yang *et al*. have shown that PKM2 directly binds to and phosphorylates histone H3 at threonine 11 upon epidermal growth factor (EGF) receptor activation. Phosphorylation of histone H3 is crucial for the removal of HDAC3 from the *CCND1* and *MYC* promoter regions, which is required for acetylation of histone H3 at K9. These processes are instrumental in EGF-induced expression of cyclin D1 and c-Myc, tumor cell proliferation, cell-cycle progression, and brain tumorigenesis [42]. They also found that PKM2 binds to c-Src-phosphorylated Y333 of β-catenin and both be recruited to the *CCND1* promoter,

leading to HDAC3 removal from the promoter, histone H3 acetylation and cyclin D1 expression, which promote cancer cell proliferation and brain tumor development [43]. In the nuclei, PKM2 also interacts with Oct-4 and HIF-1 as a coactivator, enhancing their transcriptional activity [13,44]. Simply, PKM2 regulate the balance between energy and small molecules by controlling the metabolic flux of glycolysis. At the same time, PKM2 also indirectly regulate the expression of other metabolic enzymes and cycle-related proteins by using non-metabolic function.

Clinical Applications

PKM2 as a potential therapeutic target

As an isozyme specifically expressed in cancer cells, PKM2 plays an important role in the metabolism of cancer cells. Any external factors that interfere with PKM2 will affect the metabolism of cancer cells to a significant degree. In this way, PKM2 has significant potential as a therapeutic target. Because cancer cells express PKM2 specifically, inhibition of PKM2 expression may inhibit the proliferation of cancer cells. Many studies have shown that interfering with PKM2 expression with shRNA and miRNA can both lead to cell apoptosis, reduced metabolic activity, and decreased tumorigenicity [25,45]. Other studies have shown that interfering PKM2 expression with shRNA can increase the sensitivity of cancer cells to docetaxel and cisplatin, thus promoting cell apoptosis and decreasing tumorigenicity [46,47].

PKM2 can transform between low-activity dimers and high-activity tetramers, and interfering in these transformations can change the metabolism of cancer cells. Studies have shown that when drugs are used to ensure that PKM2 exists in the form of stable dimers, the glycolytic pathway is blocked. In media containing high concentrations of glucose, proliferation of cancer cells is inhibited. However, when the medium contains glutamine, accumulation of glycolysis intermediates promotes cell proliferation [5]. Similarly, forcing all PKM2 into tetramer form can also inhibit cell proliferation, because these cells lack the precursor substances needed for cell proliferation. Hitosugi *et al*. [26] have reported that expression of Y105F mutant PKM2 in lung cancer cell line H1299 prevents the tyrosine phosphorylation of this site, and PKM2 mainly exists in the tetramer form, leading to decreased tumorigenicity. It has recently been found that many small-molecule compounds and hormones target PKM2 and can inhibit cell proliferation [48−50].

Role of PKM2 in diagnosis

Immunohistochemical results have shown PKM2 to be of diagnostic value. In tumor tissues, PKM2 shows different phenotypes at different stages. In early tumor tissues, PKM2 expression shows heterogeneity, but in metastasized

tumor tissues, PKM2 is stained strongly and homogeneously. This also indicates that PKM2 may play an important role in tumor development. When the proliferation of tumor cells that express large amounts of PKM2 is further augmented and the viability of these tumor cells is increased, the tumor becomes malignant. In addition, necrosis and renewal of tumor cells causes some PKM2s to be released into surrounding tissues. These tissues can be used in diagnosis. Studies have shown that PKM2 levels are high in the serum of patients with many different types of tumors. PKM2 also exists in the stool of gastrointestinal cancer patients and in the pleural effusion of thoracic cancer patients. PKM2 can serve as a non-organ-specific molecular marker that reflects the metabolic activity and proliferative capacity of cells [5].

Conclusion

PKM2 is regulated by many oncogenes, and serves the dual role as a switch and a speed regulator in cancer metabolism. Under the influence of many proliferative signals, the activity of PKM2 is regulated through its quaternary structure, and PKM2 can split the products of glycolysis into two

parts, one for the energy requirements and one for transformation into different precursor substances for biological synthesis. PKM2 also forms complexes with other enzymes involved in glycolysis, causing glucose to be degraded into pyruvate, which is further transformed into lactic acid and produces energy. This is critical to the growth of tumor tissues under hypoxic and anoxic conditions. The expression, translation, and activity of PKM2 are regulated by many oncogenes and metabolic intermediates so that the energy/precursor substance equilibrium of cancer cells can be realized. The fact that these oncogenes are activated during the early stage of carcinogenesis suggests that transformation from PKML/R/M1 to PKM2 is promoted. In this way, the cells acquire growth advantages and ultimately develop into leukemia or tumors visible to the naked eye.

Although Warburg first discovered the aerobic glycolysis of cancer cells almost 90 years ago, there has never been a study on the influence of glycolysis, the TCA cycle, glutaminolysis, and anabolism on overall cancer cell metabolism. It is probable that several metabolic pathways work in concert to cause the cancer cells to proliferate at the fastest speed possible. In normal quiescent cells [**Fig. 2(A)**], almost all PKM2s exist in the form of tetramers or PKM1
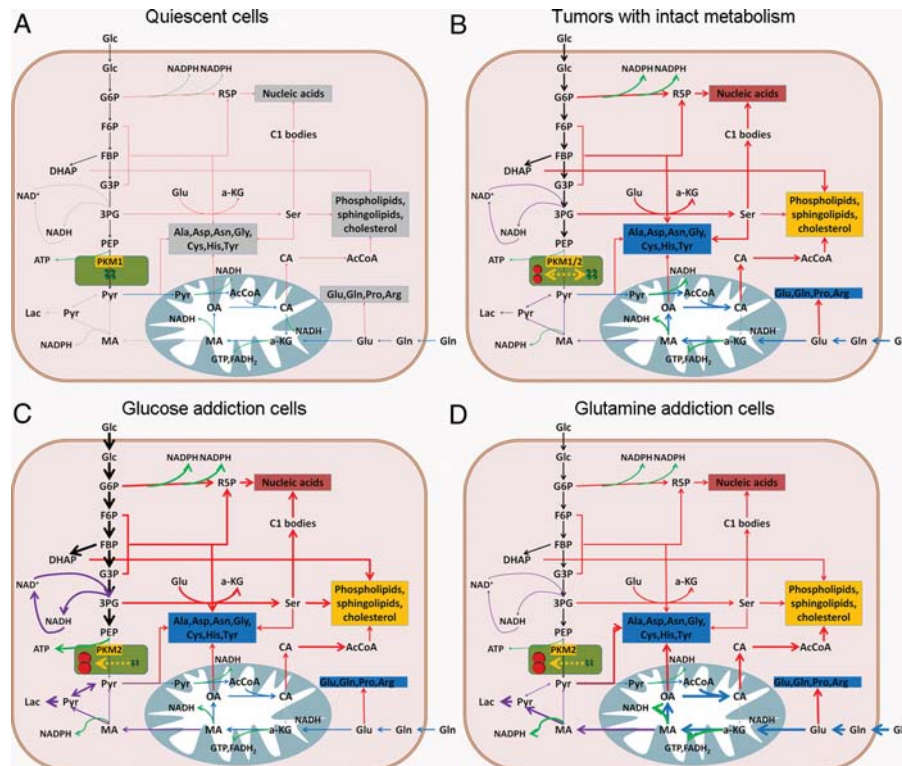
Figure 2 Metabolic profiles of normal quiescent cells and three possible types of cancer cells (A) Quiescent cells. (B) Tumors with intact metabolism. (C) Glucose addiction cells. (D) Glutamine addiction cells. The thickness of the lines and the colors of the text boxes denote the metabolic intensity; black lines denote the glycolytic pathway; blue lines denote glutamine metabolism and the TCA cycle; red lines denote anabolic pathways; purple lines denote the lactic acid pathway; green lines denote pathways that generate energy and reducing power. Glc, glucose; G6P, glucose-6-phosphate; F6P, fructose-6-phosphate; FBP, fructose 1,6-bisphosphate; DHAP, dihydroxyacetone phosphate; G3P, glyceraldehydes-3-phosphate; 3PG, 3-phosphoglycerate; PEP, phosphoenolpyruvate; Pry, pyruvate; Lac, lactic acid; OA, oxalic acid; CA, citric acid; a-KG, α-ketoglutarate; MA, malic acid; R5P, ribulose-5-phosphate.

expression, and almost all glucoses enter the complete TCA cycle, ultimately becoming fully oxidized into CO_2 and H_2O. This prevents the cells from proliferating due to a lack of material basis. Compared with normal quiescent cells, cancer cell metabolism is more diversified, and can be summed up in the following three possible metabolic spectra. In tumors with intact metabolic functions [**Fig. 2(B)**], some PKM2s are dimerized, and some glucose molecules enter the TCA cycle. Glucose serves as the carbon and energy source, and a large amount of glutamine is taken up to serve as the nitrogen source. The second type of cancer cell metabolism is as described by Warburg [**Fig. 2(C)**]. The majority of PKM2s are dimerized. Glycolytic products can generate energy and a large amount of lactic acid through PKM2. However, oxidative phosphorylation is inhibited. This type of cells can be called glucose addiction cells. In the last type of cancer cell metabolism [**Fig. 2(D)**], like the glucose addiction cells, the majority of PKM2s are dimerized. But a limited amount of glucose is used for anabolism, and glutamine is taken up in large amounts to serve as a nitrogen and energy source, thus facilitating cell proliferation. This type of cells can be called glutamine addiction cell. They can also produce lactic acid, mainly through pyruvate generated through the degradation of malic acid. This pathway meets the cancer cells' need for NADPH. Just as a single-celled organism can only survive when it displays survival advantages in the environment, in tissues, cancer cells must obey the rule of natural selection and survival of the fittest.

The use of metabolic interference to inhibit cancer cell proliferation and promote cancer cell apoptosis is an important strategy in cancer treatment. Changing the activity of PKM2 and inhibiting its expression so that either almost all or absolutely no glucose molecules entering the TCA cycle can lead to inhibition of cancer cell proliferation and even apoptosis. In this way, PKM2 has high potential as a therapeutic target. In recent years, many small-molecule compounds have been found to specifically act on PKM2 without affecting other isozymes. They all inhibit cell proliferation to a certain extent [48–50]. However, both drugs that specifically target PKM2 and drugs that have been successfully applied in clinical practice are associated with the development of drug resistance, which is probably due to the nature of targeting a single site. To focus on PKM2 and evaluate the interactions between the aforementioned metabolic pathways involved in energy generation and substance synthesis and the underlying molecular mechanisms may facilitate interference with cancer metabolism and inhibition of proliferation using multiple targets. It may prove to be an effective approach. More multi-level, multi-target investigation will be needed to further study the causes of the specificity of cancer metabolism and to translate this into effective therapies.

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