

REVIEW

Activation of the Warburg Effect by Pyruvate Kinase M2 Promotes the Occurrence and Development of Liver Cancer

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Abstract: Liver cancer, which is one of the most common malignancies, has a high incidence and case fatality rate, and is the third most common cause of death attributed to cancer. Warburg effect is a form of modified cell metabolism by which tumor cells obtain energy by glycolysis instead of oxidative phosphorylation, whether in an aerobic or anaerobic environment. It plays an important role in tumor proliferation, growth, invasion, and treatment. Pyruvate kinase M2 (PKM2), an essential key enzyme in the glycolytic process, is significantly elevated in multiple tumor tissues and plays a crucial role in the Warburg effect. Recently, increasing attention has been devoted to the mechanism of action of PKM2 in tumors, and interference with the glycolysis pathway is one of the new strategies for cancer treatment. This paper provides a review of the Warburg effect of PKM2 activation and its relationship with tumorigenesis and development and the potential value of Warburg effect in clinical diagnosis, treatment, and prognostic evaluation of liver cancer.

Keywords: Pyruvate kinase M2, Warburg effect, Hepatocellular carcinoma

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1. Introduction

Hepatocellular carcinoma (HCC) is one of the most malignant tumors, characterized by high morbidity and high case fatality rates^[1]. In China, the fatality rate of primary liver cancer has increased every year, and it is ranked second in the fatality rates attributed to malignant tumors^[2]. The occurrence and development of liver cancer involve various factors: age, sex, viral hepatitis, toxin exposure to food (aflatoxin), long-term alcohol abuse, chemical substances, and water pollution^[3]. Since the early stage of primary liver cancer tends to be asymptomatic, a confirmed diagnosis and treatment (liver resection or liver transplantation) are usually delayed^[4].

In 1956, the German biologist called Otto Heinrich Warburg and his group found that under normal oxygen conditions, tumor cells do not decompose glucose by the oxidative phosphorylation process but rely on glycolysis, which involves high-speed decomposition of glucose and low ATP yield, as the main route of energy production. This abnormal mode of energy metabolism is called the Warburg effect^[5], also known as aerobic glycolysis, which is characterized by enhanced glucose uptake and lactate production. The occurrence of the Warburg effect is closely associated with certain characteristics of tumor cells, such as changes in tumor signaling pathways, the prolonged hypoxia of microenvironment,

changes in isoenzyme profiles, and increased activity of glycolytic and glycogenic enzymes. Warburg effect plays an important role in tumor proliferation, growth, invasion, and treatment; a better understanding of aerobic glycolysis in HCC will help reveal the pathogenesis and potential therapeutic pathway of HCC.

As a key enzyme of the glycolytic pathway, pyruvate kinase M2 (PKM2) can provide metabolic raw materials such as nucleic acids, amino acids and lipids to promote the growth and division of tumor cells^[6]. In addition, increased PKM2 activity can significantly promote the Warburg effect, and is an important regulator of tumorigenesis and development^[6], closely related to the proliferation of tumor cells and invasion^[7]. However, the mechanism through which PKM2 promotes the occurrence of HCC and the role of treatment and prognosis in HCC patients remain to be elucidated. This paper analyzes the role of the Warburg effect in the occurrence and development of HCC.

2 Biological properties of the pyruvate kinase (PK)

2.1 Expression, function, and tissue distribution of PK

As a rate-limiting enzyme for the last step of the glycolytic pathway, PK is also a key enzyme for abnormal glucose metabolism in tumor cells. Within the cytoplasm, glucose eventually generates phosphoenolpyruvate (PEP) after a series of glycolytic reactions, and PEP generates pyruvate catalyzed by PK and produces ATP supplied for metabolism. In mammals, PK has four isoenzymes, including PKM1, PKM2, PKL, and PKR^[8]. PKL is mainly expressed in tissues with active gluconeogenesis, such as in the liver, kidney, and small intestine; PKR is mainly expressed in red blood cells; PKM1 is expressed in tissues with high energy consumption such as muscle, brain, heart, and skeletal muscle; and PKM2 is expressed in tissues with embryonic cells and proliferating cells, especially tumor cells (**Figure 1**)^[9,10]. Since tumor cell growth and invasion require large amounts of energy, tumor development is generally accompanied by a change in PK conformation. During embryonic formation, PKM1/L/R can replace PKM2, while in tumor cells, PKM2 increases in expression and replaces the original PK isoenzyme type; for example, during the development of HCC, by transitioning from PKL to PKM2^[7], HCC cells can increase glucose uptake levels and increase oxidative stress^[11].

2.2 Different structural characteristics and effects of PKM2

PKM2 exists in two forms: dimer and tetramer. In normal proliferating cells, PKM2 is mainly in the tetrameric form; however, PKM2 in tumor cells is mainly in the dimeric form. The two PKM2 conformations, which are mainly regulated by fructose 1,6-bisphosphate (FBP), and its

binding to PKM2 promotes PKM2 transformation into constitutive tetramers and thus increases its activity^[12]. The PKM2 tetrameric form can bind to the substrate PEP with high affinity, while the dimer form has a low affinity, resulting in high PKM2 tetramer activity at physiological FBP concentrations but with little PKM2 dimer activity^[13]. Low-activity PKM2 dimers often cause blocked glycolytic pathways followed by the accumulation of numerous intermediates in cells that are used via multiple metabolic pathways for the synthesis of biomolecules such as nucleic acids, lipids, and proteins^[12,14,15], such as glucose 6-phosphate, dihydroxyacetone phosphate, 3-phosphoglycerate, glycine, and cysteine. Phosphorylation of amino acid sites is essential for PKM2 modification. Phosphorylation of tyrosine residue 105 (Tyr105) of PKM2 results in changes in the binding site to FBP, causing dissociation from PKM2 and ultimately eliminating the allosteric effect of FBP on PKM2^[16]. It has recently been shown that viral or oncogenes such as fibroblast growth factor receptor 1, Janus kinase 2, Fms-like tyrosine kinase 3, BCR-ABL and ETS variant 6- neurotrophic receptor tyrosine kinase 3 change the phosphorylation process of PKM2 and subsequently affect tumor cell proliferation and invasion^[16,17]. The regulation of the PKM2 conformational transition by FBP and site phosphorylation allows tumor cells to change rapidly according to the different needs of the body in production capacity and synthesis of required metabolites, thus maintaining the balance between the body and tumor cell metabolism.

2.3 Selective splicing of the PKM gene

The PKM gene is approximately 32 kb, and the human, rat, and mouse PKM genes each have 11 introns and 12 exons^[18,19]. PKM1 and PKM2 are generated by variable splicing of the PKM gene with equal lengths of exon 9 and exon 10 with homology. Exon 9 is specific to PKM1, exon 10 is specific to PKM2, and only one of both exons is expressed in the correctly spliced final transcript. PKM2 production requires inhibition of exon 9 expression and activation of exon 10 expression. Three splicing factors, namely polypyrimidine binding (PTB) protein, heteronuclear protein A1 (hnRNPA1), and heteronuclear protein A2 (hnRNPA2), act downstream of oncogenic signaling, revealing exon 10 by inhibiting PKM1 expression and increasing the amount of PKM2 expression^[20-22]. However, serine/arginine-rich splicing factor 3 (SRSF3) binds to exon 10 to promote the cleavage of exon 10, thus completing the transformation of PKM1 to PKM2 and facilitating the production of mature PKM2 RNA (**Figure 2**). Since PKM1 is generally expressed only in differentiated tissues, further research has not been conducted on how mRNA splicing is regulated to increase the generation of PKM1 transcripts. Even the glioblastoma (U-118MG and A-172) and neuroblastoma (SK-N-BE) cell lines that have been used to study PKM1 expression

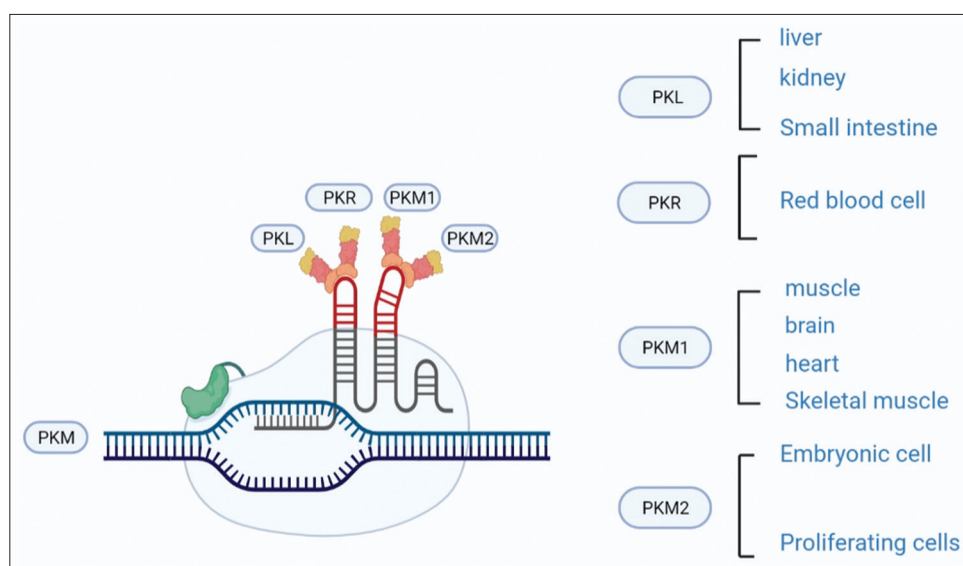


Figure 1. Distribution of different isoforms of pyruvate kinases. PKL is mainly expressed in tissues with active gluconeogenesis, such as in the liver, kidney and small intestine. PKR is mainly expressed in red blood cells. PKM1 is expressed in tissues with high energy consumption, such as muscle, brain, heart, and skeletal muscle. PKM2 is expressed in embryonic cells and proliferating cells, especially tumor cells.

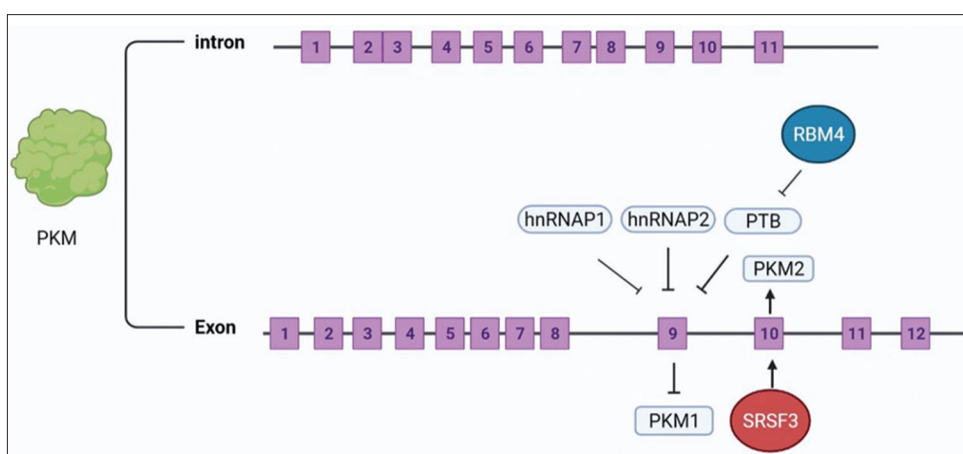


Figure 2. Selective splicing of the PKM gene. The PKM gene has 11 introns and 12 exons. Exons 9 and 10 are equal in length, and exon 9 is specific to PKM1 while exon 10 is specific to PKM2. PTB, hnRNPA1, and hnRNPA2 suppress the expression of exon 9, causing increased PKM2 production. SRSF3 binds to exon 10, thereby favoring the production of PKM 2. RBM4 splicing factor negatively regulates PKM2 expression by inhibiting the activity of the splicing regulator PTB.

predominantly express PKM2, and only 5 – 15% of the PKM transcripts are PKM1^[19,20,23]. Furthermore, it has also been found that the RNA binding motif protein 4 (RBM4) splicing factor negatively regulates PKM2 expression by inhibiting the activity of the splicing regulator PTB^[23].

3 Relationship of PKM2 with tumorigenesis and development

3.1 Association between PKM2 and tumor cell metabolism

PKM2 has two forms: a low active dimer and a highly active tetramer. The dimeric form of PKM2 is mainly expressed in tumor cells, and the tetrameric form is mainly

expressed in normal proliferating cells^[16,23,24]. Hitosugi *et al.* found that the activity of PKM2 tyrosine phosphorylation (Tyr105) is reduced when inhibiting the tetrameric form of PKM2^[16]. When Tyr105 is replaced by a phenylalanine, PK activity can be increased. The low-activity dimer form of PKM2 is an important driver of glycolysis, and instead, PKM1 and the highly active tetramers of PKM2 drive cells for the TCA cycle^[6,24]. PKM2, relative to PKM1, with higher expression and low enzymatic activity in promoting glycolysis, can promote tumor cell growth and inhibit the generation of reactive oxygen species, and the mechanisms are divided into the following two aspects^[6,24,25]. First, glycolysis can generate ATP more efficiently than oxidative phosphorylation, accelerating the utilization of carbon

for the synthesis of its bioactive material^[6]. On the other hand, low-activity PKM2 promotes the intermediates of glycolysis into the glycolytic bypass pathway, providing rich substrates for rapid proliferation of tumor cells, such as the glycerol synthesis pathway and glucose phosphate pathway, which produces NADPH and in turn inhibits reactive oxygen species generation and participates in nucleotide synthesis^[6,25]. If PKM1 replaces PKM2, it can reduce lactate production, increase oxygen consumption, reduce the tumorigenic rate of tumor cells in nude mice, and reverse the Warburg effect^[25]. The conversion of the dimer and tetrameric forms of PKM2 is regulated by many factors^[26]. FBP, as one of the intermediates of glycolysis, can be conjugated with PKM2 to promote the synthesis of tetrameric active forms; serine, derived from the conversion of glycolytic intermediate 3-phosphoglyceric acid, is also a positive regulator of PKM2 enzymatic activity^[26]. Among the negative regulators, L-cysteine also specifically inhibited the enzymatic activity of PKM2, inducing the conversion of the tetramer into a low-activity dimer^[27]. Tyrosine phosphorylation of PKM2^[28] releases FBP to convert PKM2 from the tetrameric form to the dimeric form^[29], thereby facilitating the Warburg effect in tumor cells.

3.2 Association between PKM2 and tumor angiogenesis

Tumor angiogenesis involves the proliferation of vascular endothelial tissue, as well as the destructive degradation and migration of the endothelial matrix, and can provide nutrients for the growth and infiltration of tumor cells, which is a complex process^[30,31]. Studies confirm that the dimeric form of PKM2 can promote tumor angiogenesis by promoting extracellular matrix adhesion as well as the proliferation and migration of endothelial cells, and ultimately stimulate tumor cell proliferation^[30-32]. It has been reported that the PKM2-mediated NF- κ B/miR-148a/152 feedback loop promotes tumor cell growth and angiogenesis^[33]. In some cancer cells, inhibition of miR-148a/152 expression contributes to the overexpression of PKM2, nuclear factor kappa B (NF- κ B), and insulin-like growth factor 1 receptor (IGF-IR) and plays a key role in tumor development and metabolism^[34]. Recent studies have shown that IGF-IR activation, which is an important step in tumor angiogenesis, upregulates PKM2 expression by the inhibition of miR-148a/152; PKM2-mediated enhanced binding of the hypoxia inducible factor-1 α (HIF-1 α)-p65 complex to the PKM2 gene promoter is regulated by IGF-1/IGF-IR^[33]. This binding results in the nuclear translocation of PKM2 and interacts with other molecules to regulate the expression of vascular endothelial growth factor, thereby promoting tumor angiogenesis^[34]. Thus, PKM2 plays an indispensable role in tumor cell proliferation and tumor angiogenesis.

3.3 PKM2 and tumor immunosuppression

The cells which are unfavorable may escape the immune surveillance of the body through a variety of channels, thus proliferating rapidly in the body and forming tumors. Tumor cells suppress the immune response by producing immunosuppressive molecules such as transforming growth factor beta (TGF- β), interleukin (IL)-10, indoleamine 2,3-dioxygenase or programmed death-ligand 1 (PD-L1) or by gathering regulatory T cells that can auto-secrete immunosuppressive cytokines^[35]. One of the important causes of immune evasion is PD-L1/PD-1-induced T cell failure^[35-37], and PD-L1/PD-1 blockade therapy was found to effectively improve survival in patients with cancer^[38,39], including liver cancer^[40,41]. PKM2 remodels the tumor immune microenvironment in HCC cells and promotes HCC progression by inducing the immunosuppressive microenvironment. A clear positive correlation was demonstrated between PKM2 expression and the immunosuppressive factors CD45, CD4, and CD8, and that tumors with high PKM2 expression had higher levels of inflammatory factors TNF and IL-6, the immunosuppressive factors CD274 (PD-L1), CTLA4, LAG3, and the chemokines CXCL1, and CSF1^[42]. Alternatively, in HCC cells with high PKM2 expression, infiltration of CD8⁺ T cell, Treg cell, and M2 macrophage was increased, whereas M1 macrophage infiltration was decreased^[42]. These immune cells and immune factor alterations promote the immune escape of cancer cells and effectively enhance the progression of HCC^[42]. The enhanced glycolysis enables lactate accumulation in the tumor microenvironment, improves the immunosuppressive activity in the tumor microenvironment, contributes to the immune surveillance of tumor cells to escape the host, and improves tumor survival^[43-47]. On the other hand, aerobic glycolysis coordinates chemokine molecular networks to maintain tumor immunosuppression and influence the growth of bone marrow-derived suppressive cells^[48]. These findings demonstrate that PKM2 promotes aerobic glycolysis in tumor cells (Warburg effect) and favors tumor cells from immune surveillance.

3.4 PKM2 regulates the apoptotic pathway to affect tumor growth

Goldberg *et al.* reported that silencing PKM2 expression induced apoptosis in cancer cells and inhibited the growth of tumor cells^[49]; however, the molecular mechanism by which PKM2 inhibits tumor cell apoptosis has not been deeply studied. It is well known that Bcl-2 family proteins are key regulatory proteins that determine cell fate^[50]. Bim is a type of protein and a novel member of the Bcl-2 family with multiple proapoptotic activities^[51]. The stability of Bim was shown to be regulated by protein kinase A (PKA) and differentiation-related gene 1 (Drg1)^[52,53]. Bim interacts with Bcl-xl and Mcl-1 to initiate the mitochondrial release of cytochrome c and to

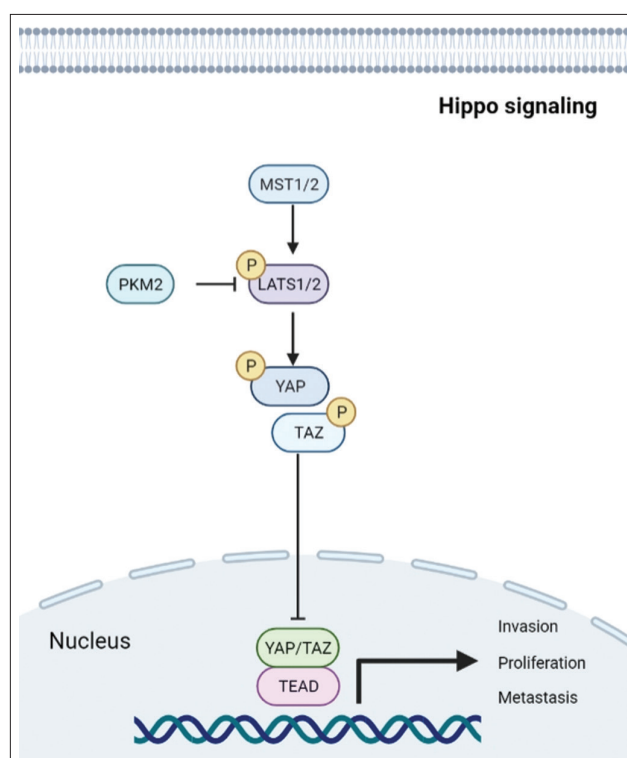


Figure 3. PKM2 regulates Hippo signaling to promote the proliferation, invasion and migration capacity of HCC cells. YAP is a downstream effector molecule of the Hippo signaling pathway. The upstream mediator LATS1/2 phosphorylates YAP and then inhibits YAP growth and antiapoptotic and cancer-promoting activities. Negative regulation of YAP and TAZ activity is the main function of the Hippo signaling pathway. PKM2 may promote the proliferation, invasion, and migration of HCC cells by inhibiting LATS1 and YAP phosphorylation and in the end affecting the Hippo signaling pathway.

activate caspase 9, thereby triggering the mitochondrial apoptotic pathway^[54]. Bim siRNA markedly abolished apoptosis induced by PKM2 depletion, and PKM2 knockdown attenuated Bim degradation, suggesting that Bim plays a critical role in PKM2 depletion-mediated apoptosis^[55]. PKM2 and Bim expression was analyzed in clinical samples of 490 HCC patients. The results showed that Bim is also an independent predictor of overall survival in HCC patients that cases with high PKM2 expression in tumor tissues are often accompanied by low Bim expression and that PKM2 expression is inversely correlated with Bim expression^[55]. Stetak *et al.* reported that PKM2 significantly induced programmed cell death in Cos-7 cells^[56]. Interestingly, Shimada *et al.* revealed that PKM2 inhibited intestinal apoptosis in epithelial cells by upregulating the antiapoptotic protein Bcl-xl^[57]. In further studies done by Kwon *et al.*, it was shown that PKM2 promotes gastric cancer cell growth by inducing Bcl-xl expression at the transcriptional level^[58]. The transcriptional activity of PKM2 has also been reported in colon cancer, suggesting that PKM2 interacts with TGIF2

and transcriptionally regulates TGF- β signaling^[59]. In conclusion, PKM2 increases the growth of tumor cells by inhibiting the apoptosis signaling pathway.

4 Mechanism of action of PKM2 in the malignant progression of HCC

4.1 Relations between PKM2 and the proliferation, invasion, and metastasis of HCC cells

Having demonstrated that PKM2 is highly expressed in HCC tissues and is closely related to the prognosis in HCC patients^[60,61], it is speculated that PKM2 also plays an indispensable role in the malignant progression of HCC cells. In 1968, Lo *et al.*^[62] first validated that PKM2 was highly expressed in rat liver cancer transplant tumors, and subsequently studies found that PKM2 was highly expressed in nuclear extracts of HCC cells^[63]. Recently, PKM2 was found to be higher in HCC tissues and lower in adjacent cancerous tissues, and high PKM2 expression is closely related to clinical characteristics such as tumor size, peritumoral vessel invasion, number of nodules, alpha-fetal protein expression, and tumor staging^[55,64]. PKM2 phosphorylates STAT3 (signal transducer and activator of transcription 3) at Y705 (tyrosine 705) to activate the transcription of HIF-1 α , which is closely related to the proliferation, invasion, and metastasis of tumor cells^[65]. Interference with the expression of the PKM2 gene can not only significantly inhibit the proliferation and migration process of HCC cells, but also promote the apoptosis of HCC cells^[66]. In the model of HCC xenograft tumor, HCC lung metastasis is greatly reduced after interfering with PKM2 expression^[11]. The substantial reduction in the migration number of HCC cell in *in vitro* studies was also due to interference with PKM2 expression^[42]. In addition, PKM2 is involved in epithelial-mesenchymal transformation (EMT) processes in HCC cells with important links to tumor cell migration, infiltration, and metastasis^[67]. These findings suggest that PKM2 plays an important role in the proliferation, invasion, and metastasis of HCC cells.

4.2 Inhibition of hippo signaling by PKM2 promotes the proliferation, invasion, and migration of HCC cells

The Hippo signaling pathway which is currently found to be a new cellular signaling pathway plays an important role in regulating tumor cell proliferation and growth. Yes-associated protein 1 (YAP) and transcriptional coactivator with PDZ-binding motif (TAZ) is the major downstream mediators of Hippo pathway, and the major function of Hippo signaling pathway is to negatively regulate YAP and TAZ activity^[68]. YAP is a downstream effector molecule of Hippo signaling pathway, and the upstream factor large tumor suppressor kinase (LATS) 1/2 phosphorylates

YAP and then inhibits the growth-, antiapoptotic-, and cancer-promoting activities of YAP^[69]. It was found that after HepG2 cells interfered with PKM2 expression, the expression of p-LATS1 and p-YAP was significantly higher in the PKM2 interference group than in the normal group while the expression of LATS1 and YAP were significantly lower than in the normal group, suggesting that siRNA-mediated inhibition of PKM2 blocked PKM2 inhibition of Hippo pathway in HCC cells and then inhibited the proliferation and invasion of HCC cells^[69]. Flow cytometry and Transwell analysis of HepG2 cell growth with LATS siRNA and PKM2 siRNA showed that LATS siRNA effectively restored the effect of PKM2 siRNA on HCC cell proliferation and restored the effect of PKM2 siRNA on the migration and invasion of HCC cells. Therefore, after interference with PKM2, Hippo signaling pathway activity is enhanced, and the inhibition of Hippo signaling activity can significantly reverse the inhibitory effect of PKM2 on the proliferation, invasion, and metastasis ability of HCC cells. PKM2 is likely to promote the proliferation, invasion, and metastasis of HCC cells by inhibiting LATS1 and YAP phosphorylation and eventually suppressing the Hippo signaling pathway (**Figure 3**)^[70].

4.3 Silencing of PKM2 activates the Epidermal growth factor (EGF)/EGF binds to receptor (EGFR) and TGF β /TGF β R signaling pathways to promote invasion and cell invasion of HCC

In epithelial cell carcinoma, the loss of E-cadherin easily leads to decreased cell adhesion, and the low expression of E-cadherin is closely associated with the low differentiation, high invasion, and high metastasis of cancer^[71]. EGF/EGFR to promote endogenous receptor tyrosine kinase activation and detachment of the E-cadherin/catenins complex on actin, thereby reducing cell adhesion capacity, affecting downstream signaling pathways, and increasing the proliferation and aggressiveness of tumor cells^[72]. After silencing PKM2 expression, E-cadherin expression decreased and N-cadherin expression increased, thereby activating signaling pathways downstream of EGF/EGFR. Phospholipase C- γ 1 (PLC γ 1) and Extracellular signal-regulated kinase (ERK1/2)^[73]. PLC- γ 1, by mobilizing actin translocation, drives the membrane into being active, which subsequently increases cell motility^[74] and also increases the proliferation, invasion, and metastasis ability of tumor cells^[75]. ERK1/2 activates matrix metalloproteinase (MMP) 1, and persistent phosphorylation of ERK1/2 can regulate MMP9^[76]. MMP1 and MMP9 can destroy the basement membrane of tumor cells and are conducive to tumor infiltration and metastasis^[77]. EMT (**Figure 4**) is an important way for cancer cells to acquire rapid growth, invasion, and metastasis, and this process can be induced by multiple cytokines. TGF is a very important inducer of EMT induction in epithelial cells^[77,78]. As a polypeptide cytokine, TGF- β promotes tumor growth, invasion, and

metastasis by evading the immune surveillance and response of tumor cells^[79]. TGF- β receptor has three different isoforms: TGF- β RI, TGF- β RII, and TGF- β RIII. TGF- β RI and TGF- β RII are transmembrane glycoproteins that are members of the serine and threonine kinase receptor families^[80]. Upon mutual binding to the TGF- β RII receptor, Upon mutual binding of TGF- β to the TGF- β RII receptor, it is recognized by TGF- β RI due to an altered conformation between the receptors, and in turn, phosphorylates TGF-RI. Receptor regulatory Smad proteins can be specifically recognized and phosphorylated by activated TGF- β RI^[81,82]. As target signaling molecules downstream of the TGF- β receptor complex, Smad proteins play an important role. It was found that the phosphorylation of Smad2/3 was increased by interfering with PKM2 expression in HCC cells^[73], and it was also found that the p-Smad2/3 levels were downregulated with TGF- β RI silencing (**Figure 4**). Therefore, it is hypothesized that in HCC cells, PKM2 can influence cellular EMT through TGF- β /TGF- β RI/Smad signaling and then regulate EGF/EGFR signaling in the form of E-cadherin dependence, ultimately enhancing HCC cell invasion and migration.

Taken together, PKM2 may induce the development of HCC by regulating the Warburg effect (**Figure 5**).

5 Clinical applications of PKM2

5.1 Application of PKM2 in tumor diagnosis

The invasion, necrosis, and metastasis of tumor cells facilitate the secretion of PKM2 into the blood, while in the patients with digestive tract tumors, PKM2 is excreted in feces. Therefore, testing the PKM2 content in the patients' blood has become an auxiliary means for the diagnosis of tumors in liver, kidney, pancreas, lungs, breast, uterus, and neck. Testing PKM2 in stool can help detect gastrointestinal tumors, such as gastric cancer and rectal cancer^[83], and also some other gastrointestinal disorders^[65]. The combined testing of CA19-9 and PKM2 also improves the detection rate of cholangiocarcinoma^[84]. Moreover, because PKM2 is difficult to detect in a normal person without tumor and can only be released into the blood from necrotic tumor cells, testing PKM2 in the blood can also evaluate the efficacy of chemotherapy and the judgment of tumor prognosis^[85,86]. Recent studies have found that highly expressed PKM2 is associated with multidrug resistance to tumors^[87]. For example, the long-term exposure of tumor cells to oxaliplatin will lead to an upregulation of PKM2 expression, which in turn causes tumor resistance to oxaliplatin; therefore, PKM2 can be used as an indicator of resistance to some antitumor drugs, such as oxaliplatin. In summary, PKM2 has the potential to be a new indicator for tumor diagnosis, tumor screening, evaluation of cancer treatment efficacy, and prognostic judgment.

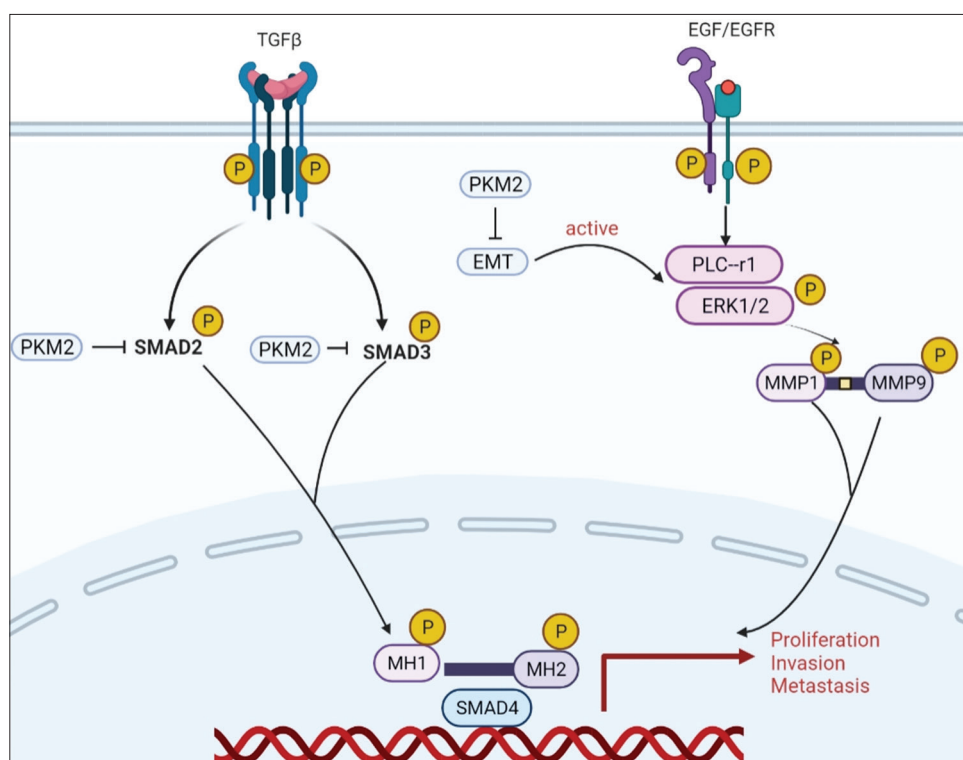


Figure 4. Silencing of PKM2 activates EGF/EGFR and TGFβ/TGFβR signaling to promote HCC cell invasion and migration. After interference with PKM2, E-cadherin expression decreases, and N-cadherin expression increases, activating EGF/EGFR signaling pathways downstream of PLCγ1 and ERK1/2. PLC-γ1 is able to activate the cell membrane and to increase the proliferation, invasion, and metastasis of tumor cells. ERK1/2 is able to activate MMP1, persistently phosphorylate ERK1/2, and regulate MMP9. MMP1 and MMP9 are beneficial to tumor infiltration and metastasis. Phosphorylation of Smad2/3 was increased after interference with PKM2 expression. Smad proteins can be specifically recognized and phosphorylated by activated TGF-βRI, p-Smad2/3 levels are downregulated after TGF-βRI silencing, and TGF-β promotes tumor growth, invasion, and metastasis by avoiding the immune surveillance and response of tumor cells.

5.2 Application of PKM2 in tumor therapy

Given the specific expression of PKM2 in tumor cells and its key role in maintaining the balance between energy metabolism of tumor cells and the supply of synthetic raw materials, intervention targeting PKM2 is an ideal strategy for tumor therapy. In 2008, adapter peptides (peptide aptamers) were studied by Spoden *et al.*^[88] to inhibit the formation of PKM2 dimers and keep PKM2 in the tetramer form, thereby inhibiting tumor cell proliferation. In 2010, Matthew *et al.*^[89] developed three novel classes of small molecule PKM2 inhibitors that promote tumor cell mortality by attenuating aerobic glycolysis, suggesting that selective targeting of PKM2 with similar drug molecules can be an effective means to treat tumors. In addition, PKM2 inhibitors that were tested in a model of mouse tumor transplantation were able to inhibit tumor growth in mice^[49]. Studies have found that multiple small molecule activators and inhibitors of PKM2, such as N-N'-diaromatic sulfamide and certain cyclulfamide thiazolane diketone derivatives, have important value in the study of PKM2 and tumor treatment applications^[89-92]. In short, targeting PKM2 treatment of tumors will become a new method for the treatment of tumors. The anti-tumor PKM2 inhibitors

have good application prospects, but the targeted treatment of PKM2 could be difficult. This is because PKM2 is not only expressed in tumor tissue, but also in normal tissue. Another reason is that gene silencing of PKM2 through small interfering RNA cannot completely inhibit tumor cell proliferation. Further experimental studies are required to study how to specifically inhibit PKM2 expression without affecting the normal cells.

6 Outlook

Warburg effect is a unique phenomenon of tumor cell metabolism, and the expression levels of PKM2 playing a key regulatory role in the Warburg effect can provide valuable therapeutic information on the therapeutic effect and prognostic evaluation. PKM2 is promising as a hallmark of early detection of HCC. Furthermore, the expression of PKM2 can be used as a new prognostic indicator after radical resection in HCC patients; therefore, these findings are important for the design of immunotherapies against PKM2. The isomer changes, transformation of localization within cancer cells, and reprogramming effects of glucose metabolism in HCC cells of PKM2 in the development of liver cancer is the focus of future

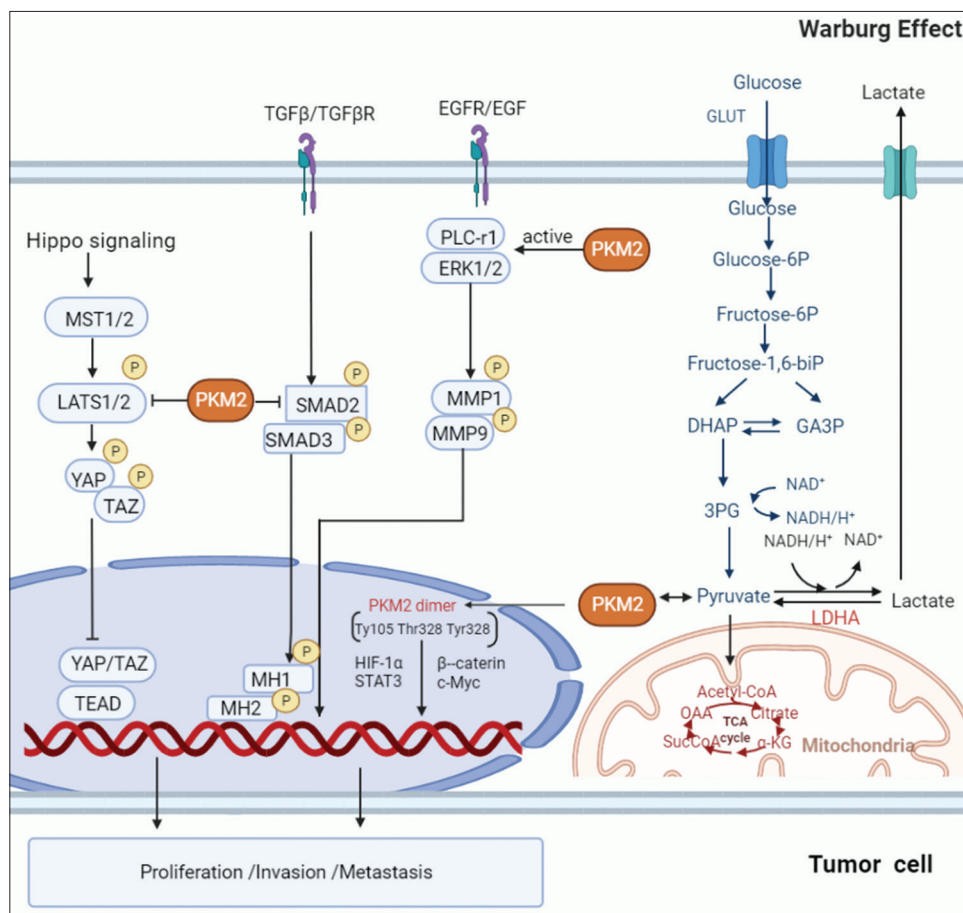


Figure 5. Regulation of the Warburg effect by PKM2 induces the development and development of HCC. The active Warburg effect of PKM2 promotes the development, invasion, and proliferation of HCC. The dimeric form of PKM2 exhibits low levels of pyruvate kinase activity that can be phosphorylated at Tyr105, Tyr328, or Thr328 and then transported to the nucleus as coactivators of certain transcription factors (e.g., HIF-1 α , β -catenin/c-Myc, and STAT3) to promote the progression of HCC. Inhibition of Hippo signaling by PKM2 promotes the proliferation, invasion, and migration of HCC cells, and silencing of PKM2 activates the EGF/EGFR and TGF β /TGF β R signaling pathways to promote the invasion and migration of HCC cells.

research, and the epigenetic changes of *PKM2* gene, such as methylation degree, expression pattern and expression of phosphorylation, acetylation, ubiquitination, etc., which may enable PKM2 to promote malignant behavior of HCC cells, have not been clarified yet. Glucose metabolism in HCC cells is an important cognitive mode of energy production in tumor cells, and the reprogramming effect of PKM2 on glucose metabolism in HCC cells highlights the need to explore the molecular mechanism of the metabolic reprogramming of HCC cells. The revelation of the mechanism of PKM2 on aerobic glycolysis (Warburg effect) in HCC cells will effectively help with the design of targeted drugs to treat HCC.

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Conflict of interest

The authors declare that this article content has no conflict of interest. All authors of this paper have read and consented to publication of the final version.

Author contributions

Y.Z., M.L. and M.Z. gathered the related literature, prepared the figures and drafted the manuscript. M.L. and M.Z. participated in the conception and design of the review. All authors read and approved the final manuscript.

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