Review Article
PKM2 affects the development of hepatocellular carcinoma

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Abstract: Hepatocellular carcinoma (HCC) is the fifth common malignant tumor worldwide and has the characteristics of insidious onset, high malignancy, invasive fast-growth, high recurrence and poor prognosis. Currently, besides the alpha-fetoprotein (AFP), there are still no other markers for the accurate HCC diagnosis. Emerging evidence shows that pyruvate kinase isozyme splice variant 2 (PKM2) is highly expressed in various tumors, and is a master regulator of cancer cell metabolism. In the occurrence and development of HCC, PKM2 is involved in a variety of signal pathways, such as Hedgehog, STAT3, PI3K/AKT and EGFR. Whether PKM2 may serve as a target for HCC diagnosis and prognosis is still unclear. Herein, we reviewed the emerging evidence about the effect of PKM2 on the HCC, aiming to push forward clinical application of PKM2 in the early diagnosis and prognosis evaluation of HCC.

Keywords: PKM2, hepatocellular carcinoma, marker

Introduction

Hepatocellular carcinoma (HCC) is the fifth common malignancy and the third leading cause of cancer related death world wide. Due to the difficulty in its early diagnosis and its poor response to available treatments, its 5-year mortality remains at a high level [1]. Up to present, alpha-fetoprotein (AFP) has been the only one marker widely used in the diagnosis of HCC. Unfortunately, the positive rate is only 70%-90% in patients with early HCC, and other diseases or conditions such as pregnancy, active hepatitis, severe hepatitis disease, etc should be excluded. Therefore, it’s imperative to develop some effective and specific markers for the early diagnosis and prognosis evaluation of HCC [2]. The therapies of HCC include surgery (including surgical resection and liver transplantation), ablation, interventional therapy, molecular targeted therapy and others. Surgical treatment of HCC is established as a potentially curative treatment modality, but the mortality is high in some patients. Local ablation is considered the first line treatment option for patients at early stages not suitable for surgical therapies, but HCC tumors in a subcapsular location or adjacent to the gallbladder have a higher risk of incomplete ablation, leading to its recurrence. In recent years, increasing attention has been paid to the molecular targeted therapy which has changed the landscape of cancer management [3]. Around 20 molecular targeted therapies have been approved during recent years for patients with breast, colorectal, non-small cell lung, renal cancer and HCC, among other malignancies [3]. Recently a multikinase inhibitor, sorafenib, has shown survival benefits in patients with advanced HCC. This advancement represents a breakthrough in the treatment of this complex disease, and proves that molecular therapies can be effective in this cancer. A better understanding of the molecular hepatocarcinogenesis is critical for identifying novel targets and oncogenic addition loops.

Currently, many studies have been conducted to investigate the molecular pathogenesis of HCC, but metabolic mechanism is rarely investigated in HCC cells. In 1926, Warburg found that pyruvate produced by glycolysis in tumor cells is
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PKM2 and its characteristic in cancer

Normal cells rely on two kinds of oxidative phosphorylation and glycolysis metabolic pathways for metabolic energy. Nevertheless, even with sufficient oxygen, tumor cells can produce large amounts of lactic acid mainly by glycolysis metabolic pathway, which is also known as Warburg effect. Originally, researchers surmised that Warburg effect is caused by mitochondrial dysfunction of tumor cells, but later found that the case is not universal. Mitochondrial function is normal in many cancer cells [8]. In aerobic glycolysis, pyruvate kinase (PKs) functions at the final step and is an important rate-limiting enzyme [9]. The existence of different PK isoforms reflects the importance of the last step of glycolysis to cope with the differential metabolic requirements of cells. The mammalian pyruvate kinase (PK) family is comprised of two genes that encode four isoforms (L, R, M1 and M2). L and R isoforms, present in liver and erythrocytes, respectively [10], are encoded by PKLR gene using different promoters. M1 and M2 isoforms are alternative splicing products of the PKM gene. M1 is mainly found in adult skeletal muscle and brain, while M2 is the dominant isoform in proliferating cells [11]. The presence of PKM2 in embryonic tissues is a first sign of its importance for proliferating cells. The PKM locus encodes for the mutually exclusive M1 and M2 isoforms of pyruvate kinase [11]. These isoforms result from alternative splicing of the PKM pre mRNA into the M1 (with exon 9) and M2 (with exon 10) isoforms. Both isoforms differ only in 22 out of 531 amino acids and the exon that is exchanged encodes for 56 amino acids [11]. This region forms the inter-subunit contact domain (ISCD) of PKM2, which is involved in the association of dimers into tetramers [12].

The distribution of the PK isoforms is tissue specific depending on the metabolic necessities of the tissues. Constitutively active M1 isoform is expressed in muscle and brain tissues.
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that need a large supply of ATP. Cells and tissues with a high rate of nucleogenesis are characterized by the expression of the isoform M2. PKM2 is the prototypic isoform of pyruvate kinase and is gradually replaced by other tissue specific isoforms during differentiation [13]. The replacement of PKM2 by other isoforms during tissue differentiation is indicative of a mechanism that ensures the strict regulation of PKM2 expression during development. Several studies have reported an isoform shift back to PKM2 during tumor formation.

In addition, the structural properties and catalytic activity of PKM2 are associated with tumorigenesis. PKM2 may exist in both tetrameric and dimeric forms. Kinetic analysis has revealed that the tetrameric form has high affinity (km, 0.03 mM) to its substrate PEP, whereas the dimeric form has low affinity to PEP (km, 0.46) [13-15]. It is proposed that the inter-conversion between dimeric and tetrameric PKM2 is dynamic. Increased levels of dimeric PKM2 that is less active than tetrameric lower the rate of glycolysis and increase the accumulation of fructose-1, 6-biphosphate (FBP) (a glycolysis intermediate product up-stream of PKM2 and an allosteric activator of PKM2), and the latter promotes the tetramerization of PKM2. Tetrameric PKM2 in turn increases the rate of glycolysis and decreases FBP concentration, leading to dimerization of PKM2. The ratio between the tetrameric and dimeric form of PKM2 decides the overall activity of PKM2 in cellular milieu [16] and thus the glycolytic flux towards catabolism or anabolism. The tetramer: dimer ratio is also regulated by post-translational modifications as well as interactions with oncoproteins which favor the inactive dimeric form of the enzyme [13]. As thus, its characteristics are closely related to many oncogenes and tumor related transcriptional factors [16] (Figure 2) and it is a bridge between cancer metabolism and tumorigenesis mechanism.

**PKM2 expression in HCC**

Studies have confirmed that PKM2 is highly expressed in HCC tissue. Immunohistochemical analysis shows that PKM2 is mainly expressed in the cytoplasm and nucleus, and PKM2 expression is higher in cancer tissues than in paracancerous tissues [17]. Same results are also observed by mass spectrometry of serum PKM2 [18]. Even more, the levels of nuclear PKM2 can predict a poor prognosis in patients with various differentiated esophageal squamous cell carcinoma [19]. However, whether the PKM2 expression can predict a poor prognosis in HCC patients is still unclear and whether combined use of AFP and PKM2 can improve the early diagnosis of HCC is needed to be further resolved.

**PKM2-related signaling pathways in HCC**

Nuclear PKM2 acts as a transcriptional coactivator and non-glycolytic functions, it functions in HCC via some important signaling pathways, including Hedghog, AKT, STAT, microRNAs, etc.
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**Effect of PKM2 on the hedgehog (Hh) signaling pathway in HCC:** Hh signaling pathway plays an important role in the process of embryonic development, which can regulate cell proliferation and differentiation. In recent years, studies have shown that Hh is not expressed in normal hepatocytes, but is related to the occurrence and development of liver cancer although the specific mechanism is still under further investigation. In HCC, when the Hh signaling pathway is activated, it can activate the expressions of Shh, Ptc, Gil-1 and Smo and other target genes or molecules (such as P21, cyclin D, etc) to participate in the proliferation and development of tumor cells. Studies also show that silencing of PKM2 can induce the down-regulation of Gil1 expression. In contrast, PKM2 over-expression can induce up-regulation of Gil1 expression. Some studies have revealed that PKM2 plays a regulatory role in the Hh signaling pathway [20]. However, evidence on the role of PKM2 in the Hh signaling pathway is still limited. In HCC, what are the upstream and downstream molecules of PKM2 in the Hh signaling pathway, whether PKM2 selectively regulates Gil1 expression, and whether PKM2 is able to regulate other molecules are needed to be further studied.

**Effect of PKM2 on AKT signaling pathway in HCC:** Many studies have demonstrated that PI-3K, AKT, and mTOR signalling pathways are regulated by dual regulation. When PI-3K and AKT are activated or PTEN and Spry2Y55F are mutated or inactivated, mTOR will be abnormally activated. Among down-regulated factors of Myc, the mTOR family and HIFs are often activated, which is related to tumor cell proliferation and nutrition supply. At present, studies have shown that HIF-1 can increase the expression of PKM, c-Myc can regulate the variable splicing of PKM mRNA, and combined HIF-1 and c-Myc can cause a high expression of PKM2, but the specific mechanism is poorly understood [21]. In PKM2 silencing H1299 and A549 cells, the p-AKT expression significantly increased, and the changes in glucose metabolism related proteins as compared to wild strains could inhibit cell proliferation [22]. Therefore, it is necessary to compare the changes in related factors of the pathway in liver cancer cell lines with those in wild strains, PKM2 silencing cells and PKM2 over expressing cells, and to investigate the consequences of PKM2 silencing and whether PI3K, AKT and mTOR inhibitors have a synergistic effect.

**Effect of PKM2 on the JAK-STAT signaling pathway in HCC:** JAK-STAT signaling pathway is involved in tumor cell proliferation, differentiation, apoptosis and immune regulation [23]. It is mainly composed of three components: tyrosine kinase related receptors, tyrosine kinase JAK and transcription factor STAT. JAK has four family members, JAK1-3 and Tyk2. STAT is known as “signal transducer and activator of transcription”. STAT plays a key role in the signal transduction and transcriptional activation. To date, a total of six members have been identified for STAT, and the biological effect of STAT3 is the most important. STAT3 phosphorylation (p-STAT3) can form homodimer or heterodimer, and enter the nucleus to regulate gene transcription. Larner et al. found that STAT3 was present in the mitochondria of cells, which affected the function of mitochondrial oxidative respiratory chain complexes I and II and STAT3 in mitochondria was important for the Ras-induced tumorigenesis [24]. In 2010, Demaria et al found that the sustained activation of STAT3 caused aerobic glycolysis and reduced the mitochondrial activity of cells [25]. Recent studies have indicated that PKM2 can regulate the development of tumor through the STAT3 signaling pathway. As in colon cancer cells, PKM2 can be used as a kinase and may phosphorylate STAT3. The p-STAT3 then activates downstream factors, such as Snail-1, Snail-2, MMP-2, MMP-9, etc. In addition, clinical findings show that PKM2 is highly expressed and STAT3 is highly activated in colon cancer [26]. However, more studies are required to verify the relationship between PKM2 and JAK-STAT pathway in HCC.

**Effect of PKM2 on β-catenin signaling pathway in cancer:** As is known to all, beta catenin (β-catenin) is a key molecule in the Wnt signaling pathway. In the study on brain tumors, epidermal growth factor receptor (EGFR) activation can induce the translocation of PKM2 into the nucleus where the PKM2 binds to K433 and phosphorylates β-catenin Y333. The resultant protein complex binds to the Cyclin D1 (CCND1) promoter region, to regulate CCND1 expression [27]. Thus, it can accelerate cell proliferation and tumor formation. In colon cancer, PKM2 may be secreted from cancer cells after activation of Wnt/β-catenin signaling.
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has a dual role: it not only plays an important role in the energy metabolism of tumor cells, but participates in the regulation of many cell signaling pathways and regulates the expressions of key genes or molecules in the tumor development. Thus, PKM2 may become a potential target in the tumor therapy (Figure 3). Although the function and regulation of PKM2 in tumor cells have been studied since 2011, information on the role of PKM2 in HCC is still limited, whether PKM2 functions through multiple or single signaling pathway needs further studies.

Effect of PKM2 on the micro-RNAs in cancer: MicroRNAs (miRs) are non-coding RNAs with specific regulatory role in gene expression. Recent reports suggest miRs have been associated with carcinogenesis and cell energy metabolism.

a. In colon cancer, miR-124, miR-137 and miR-340 can switch PKM gene expression from PKM2 to PKM1 through the feedback mechanism, which enhances oxidative stress and counteracts the Warburg effect [32].

b. In prostate cancer, results showed the miR-128-mediated ribosomal protein S6 kinase 1 (RPS6KB1)/HIF-1α/PKM2 signaling pathway can regulate the ectopic expression of SNAIL. Moreover, PKM2 can regulate cell growth, energy metabolism and play a key role in the reprogramming of cancer metabolism [33].

c. PKM2 is another target of miRNA-326. In glioma and glioma stem cells, studies found that the abnormal expression of miRNA-326 may induce cell apoptosis and impact metabolic activity by Notch pathways; PKM2 may internally adjust the expression of miRNA-326 and has the potential and trends to inhibit its expression [34].

d. Findings shown that miR-133a and miR-133b are target transcripts of PKM2, and over-

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Figure 3. Functions and regulation of PKM2 in tumor cells.
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expressed PKM2 is able to inhibit the expressions of miR-133a and miR-133b in HCC [35].

On the basis of above findings, PKM2 is associated with miRNAs and multiple signaling pathways, but their relationships need to be further studied in HCC.

PKM2-induced Warburg effect and HCC

Studies have shown that HCC cell proliferation is associated with Warburg effect instead of relying on blood vessels [36]. In the proliferation of HCC cells and nodular regeneration, anaerobic glycolysis plays an important role in the cell energy metabolism. A large number of studies have demonstrated that PKM2 is an important adjustment point. In vitro, PKM2 knockdown is able to decrease some lactic acid, which can further reduce HCC cell proliferation. In addition, Warburg effect is regulated by two transcription factors, hypoxia-inducible factor (Hifs) and C-Myc [37]. PKM2 and Hifs or PKM2 and C-Myc can cause a vicious cycle of Warburg effect. On the other hand, tripartite motif-containing protein 35 (TRIM35) can suppress the Warburg effect and tumorigenicity by blocking PKM2 Y105 phosphorylation [38], prolyl Hydroxylase3 (PHD3) can enhance the Warburg effect and promote HCC cells proliferation by hydroxylating PKM2 403/408 proline, and PKM2 K433 acetylation can phosphorylate β-catenin and promote the expression of c-Myc and Cyclin D1, indirectly leading to the recurrence of liver cancer [39].

Conclusion

Up to now, it is still difficult for diagnosis of early HCC. Clinically, besides AFP, no any another marker has been used for the accurate diagnosis and prognosis evaluation of HCC. Robust evidence shows PKM2 may become a factor used in this field: (1) PKM2 detection in the feces and plasma due to its sensitivity and specificity plays an important role in early diagnosis of colon cancer [28]; (2) PKM2 expression increases significantly in the endometrial cancer high-risk group of colon cancer patients [28]; (3) Clinical characteristics and tissue microarray assays prompted that PKM2 can predict an adverse outcome in patients with esophageal squamous cell carcinoma [19]. However, more direct evidence on the role of PKM2 in the early diagnosis and prognosis evaluation of HCC is needed.

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None.

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