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Review

Pyruvate kinase M2: A multifarious enzyme in non-canonical localization to promote cancer progression

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ABSTRACT

Rewiring glucose metabolism, termed as Warburg effect or aerobic glycolysis, is a common signature of cancer cells to meet their high energetic and biosynthetic demands of rapid growth and proliferation. Pyruvate kinase M2 isoform (PKM2) is a key player in such metabolic reshuffle, which functions as a rate-limiting glycolytic enzyme in the cytosol of highly-proliferative cancer cells. During the recent decades, PKM2 has been extensively studied in non-canonical localizations such as nucleus, mitochondria, and extracellular secretion, and pertained to novel biological functions in tumor progression. Such functions of PKM2 open a new avenue for cancer researchers. This review summarizes up-to-date functions of PKM2 at various subcellular localizations of cancer cells and draws attention to the translocation of PKM2 from cytosol into the nucleus induced by posttranslational modifications. Moreover, PKM2 in tumor cells could have an important role in resistance acquisition processes against various chemotherapeutic drugs, which have raised a concern on PKM2 as a potential therapeutic target. Finally, we summarize the current status and future perspectives to improve the potential of PKM2 as a therapeutic target for the development of anticancer therapeutic strategies.

1. Introduction

Pyruvate kinase (PK) is an evolutionary conserved metabolic enzyme that catalyzes the irreversible transphosphorylation between phosphoenolpyruvate (PEP) and adenosine diphosphate (ADP) to produce a pyruvate and ATP as the last step of glycolysis [2]. In normal cells, pyruvate is either completely oxidized to CO_2 via oxidative phosphorylation to produce more ATPs in the presence of sufficient oxygen, or converted into lactate when oxygen is low [3]. In contrast, cancer cells are well documented to rewire glucose metabolism to meet their high energetic and biosynthetic demands of excessive growth and proliferation. This metabolic reshuffle-now considered as a hallmark of cancer-involves the increased glucose uptake and enormous production of pyruvates, which instead of entering oxidative phosphorylation produce lactate without regarding the concentration of oxygen [4]. Thus, cancer cells predominantly confine ATPs synthesis to enhanced glycolysis, the phenomenon has been termed as Warburg effect or aerobic glycolysis described by Otto Warburg [5]. Cancer cells attain such a peculiar phenotype in part by overexpressing glucose transporters (Glut1 and Glut2) and glycolytic enzymes including pyruvate kinase M2 isoform [6,7].

Almost all mammalian genomes, including humans carry two distinct *PK* genes; *PKLR* and *PKM* gene, which code for the more extensively studied four PK isoforms (PKL, PKR, PKM1, and PKM2). The expression of PK isoforms is highly regulated and is tissue specific, entailing that their different kinetic properties satisfy metabolic needs of different tissues. For instance, *PKLR* gene, by using tissue-specific alternate promoter, code for the full-length PKL or PKR isoforms. PKL isoform is expressed in tissues featuring high rate of gluconeogenesis such as liver, and PKR isoform is primarily expressed in erythrocytes and to a small extent in intestine and kidneys [8]. PKM1 is expressed in other types of terminally differentiated tissues that demand a huge supply of ATPs such as muscle and brain [9]. PKM2 is predominantly expressed in highly proliferative cells with growing anabolic demands such as embryonic cells, stem cells and tumor cells in particular [9–11]. Mutually exclusive alternative splicing of pre-mRNA of *PKM* gene can generate PKM1 and PKM2 mRNAs by the inclusion of exon 9 and 10 respectively [12–14]. The molecular mechanism for alternative splicing

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of mRNA of *PKM* gene has been recently addressed. The *c-MYC* oncogene is a master regulator that control many aspects of transforming cells including the transcription of heterogeneous nuclear ribonucleoproteins (hnRNPA1, hnRNPA2) and polypyrimidine-tract binding proteins (PTBs). Subsequently, hnRNPs bind to the flanking region of exon 9 of the *PKM* gene to repress its inclusion, and thus indirectly causing the inclusion of exon 10, resulting in the expression of PKM2 in cancer cells [12,15]. In addition, a serine/arginine rich splicing factor 3 (SRSF3) can directly bind to a potent exonic splicing enhancer (ESE) site in exon 10, resulting in the inclusion of exon 10 and production of PKM2 without relying on hnRNPs intervention [16].

Previously, numerous independent studies showed that the expression shift of isoenzymes of pyruvate kinase is an essential molecular signature in many tumor types where tissue-specific isoforms (L, R, or M1) disappears, and thus replaced by PKM2 [17–19]. A tissue-specific replacement of PKM2 with PKM1 in lung cancer cells significantly inhibits aerobic glycolysis and tumor growth in nude mouse xenografts [20]. However, PKM1 and PKM2 have been recently reported to differentially co-express with high PKM2/PKM1 ratio in tumor cells supporting the Warburg effect [21,22]. *PKM2* knockdown in mouse model of breast cancer did not affect the formation of mammary gland tumors [23]. Moreover, up-regulation of both PKM1 and PKM2 isoforms and increased glycolysis have been shown in c-MYC-driven liver tumors, where depletion of PKM2 didn't affect the tumor growth [24]. Contemporary studies demonstrated that PKM2 expression is not required for tumor formation or progression in some cancers such as colon cancer and pancreatic ductal adenocarcinoma in APC-deficient and $KP^{-/-}C$ mouse models respectively [25,26]. Interestingly, these studies are consistent with meta-analyses of published datasets, which found that PKM2 expression had no effect on overall survival of cancer cells [27,28]. Hence, the current findings are not uniform with previous studies showing a complete shift from one isoform to the other one, suggesting that the overexpression of PKM2 may not be essential for tumorigenesis in all tumors, and therefore a differential analysis is needed to elucidate the precise roles of different PK isoforms in tumors of different origin.

In cancer cells, the up-regulated PKM2 at cellular and subcellular localizations exist in various oligomeric states (Box 1). Kinetic analysis revealed that the tetrameric form of PKM2 in the cytosol possess high affinity to its substrate PEP. Therefore, at physiological PEP concentration, the tetrameric form is more active (glycolytic activity) in contrast to the dimeric form that is nearly inactive [10]. Plainly, the major pool of tetrameric PKM2 is considered as cytosol of the cell. Very recently, PKM2 in dimeric/monomeric conformation has been reported in non-canonical localizations such as extracellular circulation, mitochondria and nucleus, with a number of moonlighting functions in cancer progression. Several recently published reviews have described new advances in our understanding of PKM2 functions from a “non-glycolytic” point of view [29–36]. Non-glycolytic activity of PKM2 primarily promotes cell proliferation, metastasis, angiogenesis and other cancer hallmarks (Fig. 1). In this review, we highlight recent advances regarding the PKM2 subcellular localizations, moonlighting functions in cancer progression, and the underlying mechanism of nuclear translocation induced by posttranslational modifications.

2. Secretory PKM2

Primarily detected in cytosol as a key enzyme of glycolytic complex, PKM2 has now been determined in the extracellular circulation of cancer patients with gastrointestinal, pancreatic, lung, ovarian cancer and renal cell carcinoma [37–39]. Secretory PKM2 can be detected in patient's blood sera or stool samples by using enzyme-linked immunosorbent assay (ELISA) kit, which suggests PKM2 level in the circulation as a diagnostic marker in many types of cancer [40,41]. These findings are followed by subsequent studies to understand the underlying mechanism of PKM2 secretion and its role in cancer progression.

The molecular mechanism(s) of the secretory pathways has been recently addressed that PKM2 could be possibly released in cell culture medium from the small vascular compartments; exosomes and microvesicles, which are secreted by many cell types such as B lymphocytes, dendritic cells and particularly cancer cells [42]. Secretory PKM2 has been identified in exosomes, microvesicles or as soluble proteins via mass-spectrometry-based molecular characterization of extracellular vesicles [43]. However, the secretory pathway is not fully understood, and additional study is required to identify the molecular drivers and posttranslational modifications that facilitate PKM2-microvesicles packaging in cancer cells. On the other hand, secretory PKM2 play critical roles in tumor progression. We and others identified that secretory PKM2 promotes tumor angiogenesis by facilitating endothelial cell proliferation, migration and cell adhesion to the extra cellular matrix (Fig. 1) via PI3K/AKT and Wnt/β-catenin signaling pathways [17,44]. In cancer cells, tetrameric PKM2 is like a sensor for glycolysis status and glucose demands for high proliferation. Similarly, it is possible that dimeric PKM2 signals to initiate angiogenesis reflected from the signal for nutritional requirements. In addition, Structural analysis revealed that both PKM1 and PKM2 shared almost an identical structure except for the difference of only 23 amino acids resulting from alternative pre-mRNA splicing as described above. This segment of different amino acids is mainly localized at the dimer-dimer interface, suggesting that the dimer interface is engaged in interaction with the target molecule on the endothelial cell surface and consequently promotes angiogenesis [17,45]. A subsequent study has suggested that secretory PKM2 promotes tumor cell growth and proliferation by inducing phosphorylation-mediated activation of epidermal growth factor receptor (EGFR) in an autocrine manner [46]. However, the underlying mechanism of PKM2-mediated activation of EGFR pathway is not clear.

3. Mitochondrial PKM2

Elevated level of reactive oxygen species (ROS) is a defining property of almost all type of cancer cells, which is mainly attributed to

Box 1
Oligomeric states of PKM2.

The two splice variants of *PKM* gene are PKM1 and PKM2 that exist in distinct oligomeric states. While PKM1 exists in tetrameric form and constitutively active in cytoplasm, PKM2 acquires a transitional characteristic to adopt from a highly active tetrameric to less active dimer/monomer conformation. The PKM2 tetramer-to-dimer/monomer transition is regulated by metabolites (FBP and SAICAR), amino acids (serine, cysteine, and phenylalanine), and posttranslational modifications (phosphorylation, acetylation and oxidation) (Fig. 1). The oligomeric state transition is critical for tumorigenesis, because PKM2 in tumor cells interact with and modulate the activity of various target proteins in a specific oligomeric state-dependent manner [1].

PKM2 level has been suggested as a diagnostic marker in many types of cancer. However, the subcellular fractionate of oligomeric states is not yet clear. It is proposed that to find out a clear fraction of PKM2 oligomeric forms at various subcellular localizations could improve overall diagnosis and prognosis of several types of cancer.

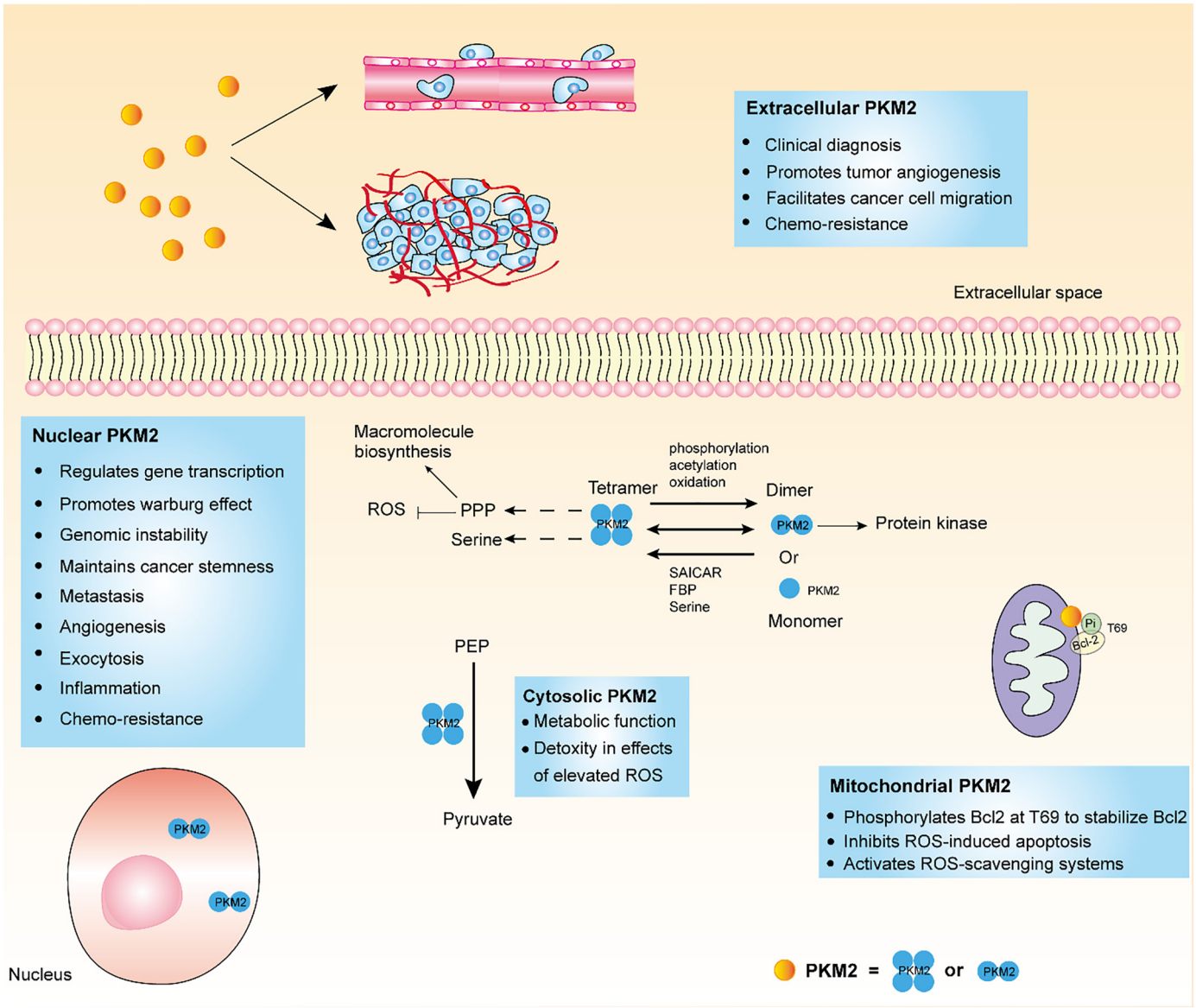

Fig. 1. Non-canonical localization of PKM2.
A schematic diagram, showing PKM2 at various identified non-canonical localizations; extracellular space, mitochondria, nucleus and actively involved in non-metabolic kinase activities to support cancer growth. The diagram also depicts the mechanism of tetramer-to-dimer/monomer transition and cytoplasmic tetrameric PKM2 with glycolytic activity.

mitochondrial dysfunction, crosstalk with immune cells, altered metabolism and increased activities of peroxisomes, oncogenes, oxidases, cyclooxygenases, lipoxygenases and thymidine phosphorylases [47]. ROS stress in normal cells often causes significant damages to cell organelles structure and function, thereby leading to the ROS-induced cellular death [48]. However, cancer cells can survive such stresses through multiple adaptations. For instance, oxidative stress induces the mitochondrial localization of PKM2 in cancer cells, where it regulates apoptosis and activate ROS-scavenging system to ensure cell survival. Firstly, Liang J and coworkers presented the possible mechanism of PKM2-mediated apoptosis. Mechanistically, under oxidative stress PKM2 translocate to mitochondria, where it interact with and phosphorylates Bcl2 at T69 to prevent proteasome-mediated Bcl2 degradation by Cul3-based E3 ligase in PKM2-dependant manner (Fig. 1), and thereby inhibits ROS-induced apoptosis [49]. Previously, numerous studies have revealed that PKM2 depletion was resulted in decreased viability and increased apoptosis in non-small cell lung cancer (NSCLC), human glioma spheroids and multiple other cancer cell lines [50–52]. Secondly, mitochondrial PKM2 activates ROS-scavenging system to cope with cytotoxicity. In cancer cells, elevated ROS level has been shown to oxidize PKM2 at C358 to inhibit its glycolytic activity. Inhibition of glycolytic activity of PKM2 literally diverts glucose flux into the PPP to generate sufficient reducing potential (NADPH + H^+) for the detoxification of ROS. These findings suggests that mitochondrial PKM2 promotes tumorigenesis by dealing with apoptosis and cytotoxicity due to elevated ROS level [53]. Likewise, methylated PKM2 localized to the mitochondrial-associated endoplasmic reticulum membrane (MAM) can also reshuffle metabolic phenotype in cancer cells. Thoroughly, PKM2 is methylated by a co-activator-associated arginine methyltransferase-1 (CAMR1) at arginine residues R445, R447 and R455. The methylated PKM2 translocate to MAM, where it interacts with and downregulate the expression of inositol 1, 4, 5-trisphosphate receptors (InsP3Rs). The reduced expression of InsP3Rs leads to decrease mitochondrial membrane potential and Ca^{2+} uptake, which is essential for activating pyruvate dehydrogenase (PDH) in Ca^{2+}-dependent manner to support oxidative phosphorylation. Thus,

methylated PKM2 play an important role in promoting tumor cell proliferation, migration and metastasis by reprogramming oxidative phosphorylation to aerobic glycolysis [54].

4. Nuclear PKM2

4.1. Nuclear PKM2 regulates gene transcription and promote cell proliferation

A multitude of studies have indicated that dimeric PKM2 exists in nucleus of cancer cells, where it functions as a co-activator of several transcription factors to modulate the expression of target genes, which subsequently contribute to aberrant metabolism and tumor growth under different physiological and pathological circumstances [20,55]. For example, in hypoxic conditions, hypoxia-inducible factor 1 (HIF-1) promotes PKM2 activation and nuclear translocation to interact directly with HIF-1α subunit. The PKM2-HIF-1α complex promotes the transactivation of HIF-1 target genes, including glucose transporter 1 (*GLUT1*), lactate dehydrogenase A (*LDHA*), and pyruvate dehydrogenase kinase 1 (*PDK1*) [56]. Similarly, EGFR induces PKM2 accumulation in the nucleus, where it interacts with c-Src-phosphorylated Y333 of β-catenin. The PKM2-β-catenin axis binds to the *CCND1* promoter to remove HDAC3 and phosphorylates histone H3 at T11, which is required for both the histone H3 acetylation at K9 and the expression of *CCND1* and *MYC* genes. In human glioblastoma cells, PKM2-mediated β-catenin transactivation has been shown to enhance the c-MYC expression, which in turn upregulates the glycolytic enzymes, including PKM2. These findings conclude that nuclear PKM2 promotes aerobic glycolysis and cell proliferation in a positive feedback mechanism [57–59]. Furthermore, nuclear PKM2 as a protein tyrosine kinase directly phosphorylates the signal transducer and activator of transcription 3 (STAT3) at T705, thereby contributing to the transcription of many STAT3 target genes, including mitogen-activated protein kinase kinase 5 (*MEK5*) to drive cancer cell proliferation [60].

4.2. Role of nuclear PKM2 in genomic instability

In cancer cells, PKM2 has been suggested as a novel modulator associated to genomic instability, an evolving hallmark of cancer which endows a newly transformed cell to acquire other essential hallmarks [61]. Despite the complex underlying molecular mechanisms, the inactivation of genome guardian *p53* gene is believed to be a key process in occurring genomic instability. Primarily, p53 regulates mitotic checkpoints and the cells lacking the p53 activity are inferior in response to DNA or chromosomal damages, subsequently carry the potential oncogenic mutations [62]. Li et al. demonstrated that nuclear PKM2 in cooperation with other active partners (p53 and H2AX) promotes genomic instability in response to DNA damage in tumor cells. Firstly, PKM2 interacts directly with p53 protein to inhibit p53-dependent transactivation of *P21* gene, resulting in the nonstop G1 phase in cancer cells being exposed to DNA damaging [63]. Secondly, DNA-damaging stimulus induces direct interaction between PKM2 and histone H2AX, which results in the PKM2-induced phosphorylation of H2AX at serine residue S139 to generate γ-H2AX. This process is an initial and sensitive marker of DNA double strand breaks [64]. The current findings indicate that nuclear PKM2 with its kinase activity promotes genomic instability following DNA damages.

4.3. Nuclear PKM2 maintains cancer stemness

In 2014, Steven WL reviewed the previous data since Schofield's original article [65], and hypothesized that the extracellular matrix, secretory proteins, inflammation status, physical parameters and environmental signals such as hypoxia could be the key components of tumor microenvironment in determining the fate of cancer stem cells (CSCs) [66]. More recently, several studies showed that PKM2 with protein kinase activity maintains cancer stemness, which generates various lineages of cancer cells. PKM2 interacts with EGFR to induce stemness-related genes, including ATP binding cassette subfamily G member 2 (*ABCG2*), kruppel like factor 4 (*KLF4*), aldehyde dehydrogenase 1 (*ALDH1*) and *c-MYC,* while silencing of either *PKM2* or *EGFR* significantly decreases the stem-like properties and inhibits tumorigenesis in vivo and in vitro, thereby supporting the statement that PKM2 expression upregulates the stemness-related genes in nuclear EGFR-dependent manner [67]. PKM2 has been previously reported to interact with octamer-binding transcription factor 4 (OCT4; a major regulator of cell pluripotency) and regulates the OCT4-mediated transcriptional activities in phosphorylation-independent manner [68]. Moreover, glucose restriction stress activates adenosine monophosphate-activated protein kinase (AMPK) in cancer cells, which induces nuclear translocation of PKM2 mediated by Ran proteins, where PKM2-OCT4 interaction accelerates the expression of OCT4-target genes to enrich the CSC population and facilitate them to survive glucose restriction stress [69]. Inconsistently, one previous report has indicated that dichloroacetate-induced nuclear PKM2-OCT4 interaction significantly reduced the OCT4 role in maintaining stemness, and promoted cell differentiation and sensitivity to death in glioma cells [51]. These studies suggest that PKM2-OCT4 complex and its role in maintaining stemness is based on different cellular milieus and stress conditions. Tyrosine kinase screening identified multiple kinases frequently activated in different types of cancer. Such kinases are shown to phosphorylate PKM2 at Y105 to exert oncogenic functions in part via activating the yes-associated proteins (YAP) and its downstream signaling to enhance cancer stem-like cell properties [70]. In brief, the current research suggests that nuclear PKM2 functions as an interacting partner of various proteins of tumor cells to play a crucial role in maintaining cancer stemness, and thereby clarifies the paradox of PKM2 multifarious nature in tumorigenesis (Fig. 2).

4.4. Nuclear PKM2 and cancer metastasis

Metastasis is the most pestilent among all cancer hallmarks, and it is strongly believed that more than 90% of cancer mortality associated with solid tumors is due to metastatic spread of the primary tumor to the remote areas of normal tissues, where they have potential to initiate the secondary tumor [71]. Growing evidence suggests that PKM2 is exclusively overexpressed in cancer cells and play a key role in tumor growth, invasion, and metastasis [20,72]. Epithelial-mesenchymal transition (EMT) is the most crucial feature of metastatic cancers which stimulates nuclear translocation of PKM2 to inhibit E-cadherin activity. In details, EMT-stimulated nuclear PKM2 interacts with TGF-β–induced factor homeobox 2 (TGIF2) and recruits HDAC3 to the *CDH1* gene (encodes E-cadherin) promoter, results in the deacetylation of histone H3 and down-regulation of *CDH1* gene. Thus, PKM2-mediated down-regulation of E-cadherin lead to the loss of epithelial cell-cell adhesion, and subsequently promotes the invasion and metastasis [73]. Wei Wang and co-workers published several articles, demonstrating that altered expression of various small molecules such as manganese superoxide dismutase (SOD2), miR-138, miR-222, and miR-181a significantly influenced invasion and metastasis in tongue squamous cell carcinoma (TSCC), via the signaling pathways including miR-138-Slug, the SOD2-H_2O_2 and the extracellular signal regulated kinase (ERK)-Slug pathways [74–78]. Subsequently, the same group of researchers, re-considered the role of PKM2 in the development and metastasis of TSCC. They revealed that miR-138-mediated EMT stimulates the PKM2 nuclear translocation, where it enhanced the metastatic potential of TSCC through the miR-138-PKM2 pathway [79]. Our laboratory reported that STAT3 is an essential factor for PKM2-induced metastasis. The protein kinase activity of the PKM2 dimer is required for STAT3 up-regulation and nuclear translocation to promote cell migration and adhesion in colon cancer [80]. In addition, AKT2-PKM2 has been shown to regulate the cellular migration/invasion in ovarian cancer and promote the

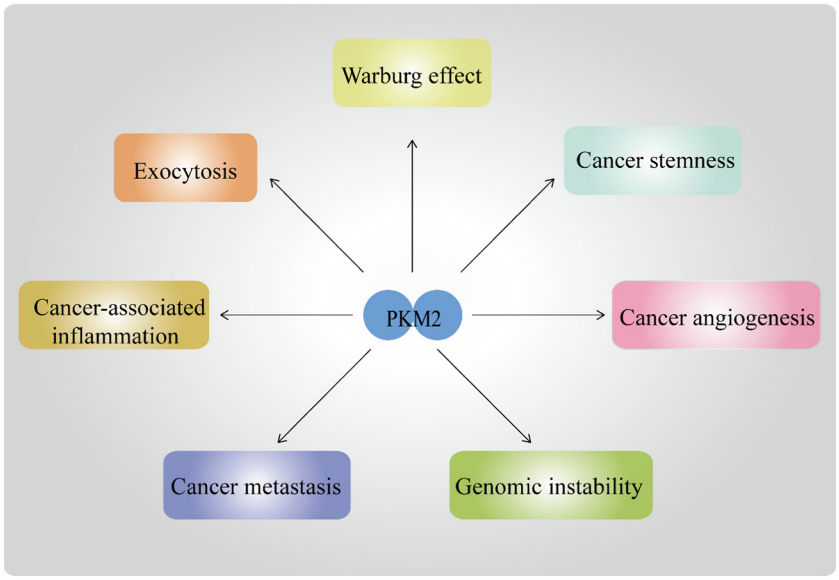

Fig. 2. Moonlighting functions of dimeric PKM2.
Dimeric PKM2 at various sub-cellular localization plays a vital role in multiple cancer hallmarks, and thus promotes tumorigenesis.

metastatic potential in lung cancer via AKT2-PKM2-STAT3-NF-κB p65 axis. Thoroughly, EGF stimulates protein kinase Bβ (AKT2) expression and its nuclear translocation. AKT2-induced PKM2 expression up-regulates STAT3 transcription and promotes NF-κB p65 nuclear translocation, suggesting that AKT2 mediates cancer metastasis in a PKM2-dependent manner [81].

4.5. Nuclear PKM2 and tumor angiogenesis

Cancer is basically a condition of uncontrolled growth, infinite cell division and less prone to apoptosis, and therefore requires an extra supply of oxygen and nutrients. The rebellious cancer cells tackle with these circumstances via acquired hallmarks such as altered metabolism and angiogenesis [61]. Tumor angiogenesis is the formation of new blood vessels from a pre-existing vasculature, which has been considered for decades as a hot topic in cancer research. Over the last decade, many studies have focused on the potential molecular drivers that facilitate the tumor angiogenesis [82,83]. IGF-IR activation is now considered as a common event in human cancer. Two recent studies on PKM2 potential role in tumor angiogenesis demonstrated that IGF-1/IGF-IR induces HIF-1α interaction with NF-κB subunit p65/RelA in response to hypoxic condition. The HIF-1α-p65 complex binds to PKM2 promoter region, leading to PKM2 upregulation and nuclear translocation, where it functions as a protein kinase alone or through interactions with other factors to regulate the vascular endothelial growth factor (VEGF) expression and secretion in endothelial cells, thereby regulating tumor angiogenesis [84,85]. However, an additional study is needed to evaluate how PKM2 interacts with its target molecules on the surface of epithelial cells and the underlying mechanism that how PKM2 facilitate the cell migration and adhesion to ECM.

4.6. Nuclear PKM2 and exocytosis

Exocytosis is an essential phenotype of all cell types, which is predominantly exploited for the intercellular communication via secretory vesicles. In extracellular fluid, the essentially known two types of secretory vesicles are: exosomes (50-100 nm) and ectosome (100-500 nm) also referred as micro-vesicles (MVs) and apoptotic bodies [86]. Tumor cells actively secrete exosomes and ectosomes to communicate with extracellular microenvironment, and their role in promoting tumorigenesis is increasingly appreciated [87,88]. The molecular drivers responsible for exocytosis and the underlying mechanism for the secretion of exosomes is an emerging area in cancer research. The upregulated PKM2 in tumor cells is phosphorylated by various norms which could be a key regulator of exosome secretion. PKM2 has been shown to phosphorylate the synaptosome-associated protein 23 (SNAP-23) at S95 via its protein kinase activity. SNAP-23 in turn enables the formation of the N-ethylmaleimide-sensitive fusion factor attachment protein receptor (SNARE) complex to allow exosomes release [89]. Like many other cells, tumor cells also employ the soluble SNARE complex that may help in the exocytic release of exosomes [90].

4.7. Nuclear PKM2 as a new player in cancer-associated inflammation

Inflammation has been linked to cancer back in the 1870s, when Rudolf Virchow noticed WBCs in cancer tissues for the first time [91]. Inflammation and cancer have several common phenotypes such as enhanced proliferation, cell survival, angiogenesis and cell migration, which are regulated by growth factors, proinflammatory cytokines and proangiogenic factors [92]. In addition, both the activated immune cells (e.g., macrophages, dendritic cells and T cells) and cancer cells display Warburg effect to attain efficient energy for cellular process such as to produce proinflammatory mediators, cytoskeleton rearrangement and cellular proliferation. Moreover, Warburg effect or metabolic shift in immune cells contribute to the innate immune functions [93,94]. Growing evidence suggested PKM2 as a new player to regulate both the immune cells metabolism and genesis of inflammation.

PKM2 as a co-activator interacts with HIF-1α and activates the transcription of glycolysis-related genes in Lipopolysaccharides (LPS)-activated macrophages, which results in the excessive lactate production, hyperacetylation of high mobility group box 1 protein (HMGB1) and its released from activated macrophages. Furthermore, PKM2-HIF-1α axis in LPS-activated macrophages directly binds to IL-1β promoter to modulate the IL-1β production, macrophage polarization, glycolytic reprogramming and Warburg metabolism. These findings suggest that PKM2 is a critical player in regulating inflammatory responses via metabolic reprogramming [95,96]. Concurrently, our laboratory demonstrated that PKM2 may regulate the production of TNF-α and IL-1β via NF-κB-PKM2-STAT3 pathway, suggested PKM2 as a key mediator to modulate the cytokines production in inflammatory microenvironment and tumor progression [97]. PKM2 with protein kinase activity also

regulates the NLR family pyrin domain containing 3 (NLRP3) and absent in melanoma 2 (AIM2) inflammasomes activation by modulating eukaryotic translation initiation factor 2 alpha kinase 2 (EIF2AK2) phosphorylation in macrophages. Pharmacological and genetic inhibition of the PKM2–EIF2AK2 pathway attenuates activation of NLRP3 and AIM2 inflammasomes and limits the release of IL-1β, IL-18 and HMGB1 in vitro or in vivo [98]. The current studies provide a novel insight into the mechanism underlying the metabolic control of inflammation, support a close link between PKM2-mediated inflammation and cancer, and highlight the importance of targeting aerobic glycolysis (especially PKM2) for the development of anti-inflammatory and anti-cancer therapies.

5. Mechanism of nuclear translocation of PKM2

5.1. PKM2 posttranslational modifications

Posttranslational protein modification (PTM) is the most efficient regulatory mechanism of cellular proteins that influence their activity, interaction with other molecules and subcellular localization. PKM2 is harboring numerous conserved PTM sites that remarkably modulate its structural and functional properties such as oligomeric state, catalytic activity and binding of allosteric activator. These modulations induced by various PTMs allow the subcellular localizations of PKM2 in response to different exogenous or endogenous stimuli such as unrestrained growth factors, increased glucose level and ROS concentration, which cancer cells encounter during tumor initiation or maintenance [20,53,99,100]. In this part of the review, we describe the PTMs exclusively induced the PKM2 nuclear translocation and their functional consequences in tumorigenesis (Fig. 3).

5.1.1. Acetylation

Acetylation play an important role in determining the nuclear localization and oncogenic function of PKM2. Human PKM2 harbors two key acetylation sites (K433 and K305) that modulate PKM2 activity in different ways in response to different stimuli. On the one hand, acetylation at K433 mediated by acetyltransferase p300 occurs in response to a spectrum of mitogenic and oncogenic signals, which distress the PKM2 binding affinity to its allosteric activator called fructose 1, 6-bisphosphate (FBP). Thus, K433 acetylation promotes PKM2 dimerization, its nuclear localization and protein kinase activity to phosphorylate STAT3, histone H3 and their downstream genes [15,101]. On the other hand, tumor cells strategically increase the glucose uptake, which induce the PKM2 acetylation at K305 mediated by acetyltransferase P300/CBP-associated factor (PCAF). However, this sort of acetylation promotes the PKM2 interaction with a constitutively active chaperone protein the heat shock 70 (HSC70), and thereby promote the lysosome-dependent degradation via chaperone-mediated autophagy. As a result, the reduced expression of PKM2 cause to accumulate the upstream glycolytic intermediates, including FBP, G6P, and to shunt the glycolytic flux into the PPP for the biosynthesis of nucleotides and amino acids to promote tumor growth [100].

5.1.2. Succinylation

Succinylation is a newly identified PTM that involves the addition of succinyl group to the lysine residue of wide variety of proteins. Recently, PKM2 has been proposed to undergo succinylation catalyzed by succinyltransferases and desuccinylation catalyzed by SIRT5, which is a member of sirtuin family of HDACs. Unlike other family members that exhibit NAD^+-dependent protein deacetylase activity, SIRT5 catalyzes lysine desuccinylation of PKM2 [102,103]. Succinylation of PKM2 at two lysine residues (K311 and K498) play an important role in modulating its enzymatic activity. For example, succinylation at K311 reduces pyruvate kinase activity, and promotes the tetramer-to-dimer transition, nuclear translocation and protein kinase activity, which regulates the IL-1b induction in LPS-induced macrophages. Moreover, SIRT5 directly interacts with and desuccinylates PKM2 at K311 to suppress PKM2-mediated pro-inflammatory responses in macrophages [103]. Another study demonstrated that ROS-induced SIRT5 interacts with and desuccinylates PKM2 at K498 to inhibit its activity. This inhibition of PKM2 in tumor cells may redirect the glucose metabolites into the pentose phosphate shunt to produce sufficient NADPH, thereby supporting the cell survival and proliferation under acute oxidative stress [104]. These findings suggest that the dynamic regulation of SIRT5-dependent succinylation/desuucinylation is critical in modulating PKM2 activity to support cell survival and proliferation. However, what cellular circumstances induced the PKM2 succinylation and how cells balanced the total PKM2 succinylation/desuccinylation level to support cell survival and proliferation are compelling questions to be answered.

5.1.3. Sumoylation

Sumoylation is another important PTM where a class of enzymes covalently attach a small ubiquitin-like modifier (SUMO) to the target proteins in an ATP-dependent enzymatic cascade that resembles ubiquitylation. Sumoylation is widely involved in protein modifications that influence many aspects of target proteins, including their interaction with other proteins, DNA or RNA [105]. Growing evidence suggest a link between sumoylation and tumor development. A study by Spoden and collogues reported that PKM2 is a potential sumoylation target [106]. The PKM2 interaction with SUMO–E3 ligase PIAS3 (protein inhibitor of activated STAT3) induce sumoylation and altered its binding affinity for allosteric substrate FBP, thus inhibits PKM2 glycolytic activity and promotes nuclear translocation [106]. More recent report evidenced PKM2 sumoylation at lysine residue K336 catalyzed by SUMO1, which promotes aerobic glycolysis and cell proliferation in cancer cells, however K336 sumoylation leads to nuclear translocation remains ambiguous [107].

5.1.4. Phosphorylation

Aberrant oncogenic protein kinases phosphorylate PKM2 at tyrosine, serine and threonine residues to modulate its glycolytic and non-glycolytic functions. Tyrosine phosphorylation inhibits the pyruvate kinase activity of PKM2, which directly rewires the glycolytic intermediates into biosynthetic metabolism to support tumor growth [99]. What we will discuss herein concerns the serine/threonine phosphorylation that supports the nuclear translocation and moonlighting functions of PKM2.

EGF-stimulated cancer cells recruit EGFR that triggers the extracellular signal-regulated kinase1/2 (ERK1/2) to phosphorylate PKM2 at serine residue (S37). The ERKs specifically phosphorylates PKM2 but not PKM1, since ERKs substrates predominantly express a docking domain followed by LXL motif, which is exclusively expressed by PKM2-specific exon 10. The phosphorylated PKM2 recruits peptidyl-prolyl isomerase NIMA-interacting 1 (PIN1) that exposed the PKM2 sterically masked nuclear localization signal (NLS) site to allow its binding to importin α5. Subsequently, PIN1 and importin α5 collaboratively promotes the PKM2 nuclear translocation, where it interacts with β-catenin to induce the expression of c-MYC, which in turn induces the expression of downstream glycolytic enzymes. In short, EGFR-stimulated S37 phosphorylation of PKM2 promotes its nuclear translocation to support metabolic reprogramming in cancer cells [59,108,109]. Insulin growth factor 1 (IGF1)-induced AKT in the cytosol directly phosphorylates PKM2 at another serine residue (S202) to promote its nuclear translocation, where it function as a co-activator of STAT5A transcription factor to induce the expression of cyclin D1, which functions downstream to the IGF/PI3K/AKT signaling pathway to promote tumor growth [110]. Finally, PKM2 phosphorylation at threonine T454 has been shown to promote its nuclear translocation and non-glycolytic function such as the transcriptional co-activation of HIF-1α and β-catenin to support cancer growth and drug resistance. Threonine phosphorylation is mediated by an oncogenic proviral insertion in murine

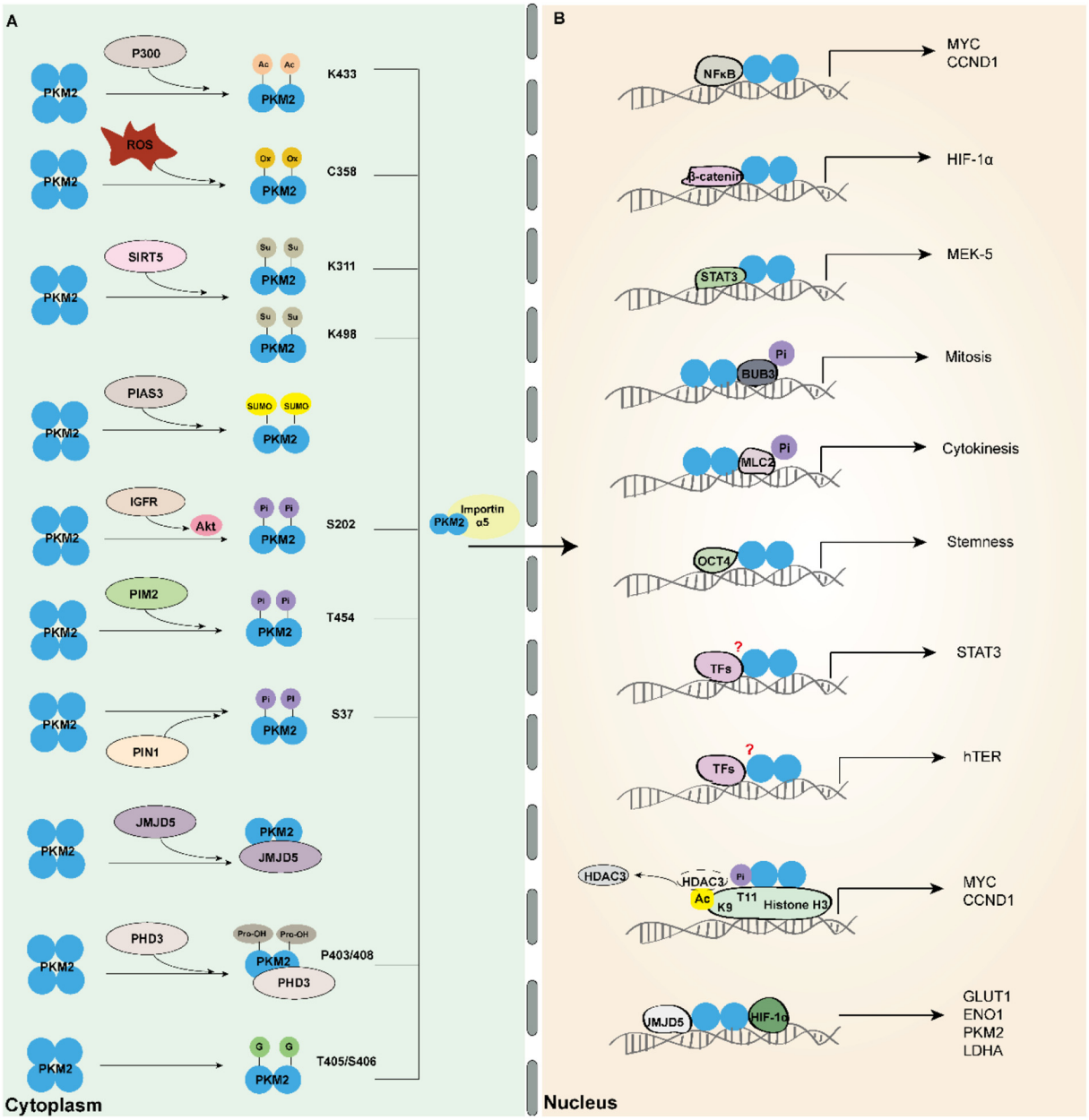

Fig. 3. Nuclear translocation of PKM2:
(A) Cytoplasmic PKM2 after PTM via acetylation, succinylation, sumoylation, phosphorylation, hydroxylation, oxidation, O-GlcNAcylation and translocate into the nucleus of cancerous cell with the assistance of carrier proteins such as importin α5 (B) In the nucleus, PKM2 with its protein kinase activity serve as a transcription co-activator to regulate various genes expression and signaling pathways linked to the cancer progression.

lymphomas 2 (PIM2), which is a serine/threonine protein kinase [111].

5.1.5. Hydroxylation

Protein hydroxylation catalyzed by 2-oxoglutarate-dependent dioxygenases has been established as an efficient posttranslational modification, which introduces a hydroxyl group (-OH) into a proline, lysin, asparagine and histidine residues of target proteins. Hydroxylation primarily influence the stability, kinase activity and protein-protein interaction of substrate proteins. In cancer cells, proline hydroxylation is considered more crucial in regulating numerous cellular process including oxygen-response pathways [112,113]. For example, PKM2 hydroxylation at proline residues (P403, P408) catalyzed by prolyl hydroxylase 3 (PHD3) induces the PKM2-HIF-1α interaction, which in turn recruits p300-acetyletransferase to promote the transactivation of HIF-1α target genes. However, reduced lactate production and increased oxygen consumption rate (OCR) were observed when PHD3 was knocked down in cancer cells [56]. The following study demonstrated that Jumonji C domain-containing dioxygenase (JMJD5; a demethylase/hydroxylase enzyme) destabilizes the PKM2 tetrameric assembly and promotes the nuclear translocation, where it induces the transactivation of HIF-1α target genes in a similar way to PHD3-dependent hydroxylation [114]. Nevertheless, JMJD5-mediated hydroxylation and nuclear translocation of PKM2 remains to be elucidated.

5.1.6. Oxidation

Regulation of endogenous ROS levels is essential for proper functioning of normal cells [115]. Cancer cells, however experience an elevated ROS level that impact tumor development and progression in multiple ways. For instance, increased ROS concentration lead to the ROS-mediated oxidation of PKM2 at cysteine residue C358 as described

earlier (Section 3), which reduce its glycolytic activity and induce the tetramer-to-dimer transition. Thus, PKM2 oxidation reshuffle the glucose flux into anabolic pentose phosphate pathway, thereby producing the sufficient reducing potential for ROS detoxification, subsequently facilitates cell proliferation and cancer progression [37,53]. The mechanism is poorly understood, and if ROS-mediated PKM2 oxidation lead to the nuclear translocation is still unknown.

5.1.7. O-GlcNAcylation

Protein glycosylation involves the covalent attachment of a diverse set of sugar moieties. In mammals, these modifications are categorized into three sub-types; N-linked, O-linked, and C-linked glycosylation. O-linked protein glycosylation (O-GlcNAcylation) is catalyzed by O-GlcNAc transferase (OGT), which involves the addition of β-*N*-acetylglucosamine (O-GlcNAc) moieties to the hydroxyl group of serine or threonine residues of cytoplasmic, nuclear and mitochondrial proteins [116]. O-GlcNAcylation may significantly impact tumor progression, by modulating metabolic reprogramming of cancer cells [117]. A study by Wang et al. demonstrated that O-GlcNAcylation of PKM2 at threonine (T405) and serine (S406) residues significantly modulates both metabolic and non-metabolic functions by altering its oligomeric conformation. Firstly, O-GlcNAcylation disrupts the tetramerization interface and reduces the glycolytic activity, which directly shunt the glucose flux toward the anabolic pathway to support cell proliferation. Secondly, the destabilization of PKM2 tetramers leads to the exposed NLS site as well as downstream S37 phosphorylation, thereby favors the accumulation of dimeric PKM2 in the nucleus, where it regulates c-MYC target genes [118].

6. Therapeutic potential of PKM2

Targeting cancer metabolism is an emerging avenue in cancer drug discovery. PKM2 is a crucial metabolic enzyme of multifarious nature, and is associated with other oncogenes, tumor suppressors, signaling pathways and crosstalk at cellular and subcellular level to support tumorigenesis [119]. Therefore, PKM2 could be a potential therapeutic target in intervening cellular metabolism in cancer treatment.

6.1. PKM2 inhibition

PKM2 inhibition could lead to reduced glycolysis and energy regeneration, promote apoptosis and improved drug sensitivity in cancer cells. Several small inhibitor molecules have been identified on that account, including but not limited to shikonin and its analogs, flavonoids derivatives and 2, 3-didithiocarbamate substituted naphthoquinones which, by binding to allosteric site of PKM2 inhibit its activity and lead to reduced glycolysis in cancer cells [120–124]. Scutellarin, a representative flavonoids derivative, has been shown to directly targets PKM2 to promote its nuclear translocation, where it induces G2/M arrest by downregulating the expression of cyclin-dependent kinase 1 (CDK1) and cyclin B in Hela cells [125]. In addition, there is evidence showing that siRNA specifically targets *PKM2* at mRNA level and induces the caspase-mediated apoptosis in tumor cells [50].

Cancer cells have the ability to develop resistance against a wide range of anti-cancer drugs, and PKM2 play a crucial role in such resistance acquisition processes. More recently, data have emerged indicating that PKM2 inhibition could improve drug sensitivity in cancer cells. For instance, Chen WP and his colleagues showed that miR-122 significantly inhibits PKM2 activity to reverse the doxorubicin-resistance and induce apoptosis in Huh7 cancer cells [126]. CD44 interacts with PKM2 and suppresses its activity via increasing the PKM2 phosphorylation at threonine (T105) residue, whereas CD44 ablation rewires aerobic glycolysis into mitochondrial respiration and increases ROS production, which significantly enhance the cisplatin sensitivity in colorectal cancer cells [127]. Thus, cisplatin resistance can be surmounted successfully by obstructing PKM2 activity in various cancer types where cisplatin is applicable. Gefitinib, a tyrosine kinase inhibitor (TKI), has been approved as a monotherapy treatment for non-small cell lung cancer [128]. A study demonstrated that PKM2 also participate in gefitinib resistance by activating STAT3 pathway, while both PKM2 knockdown and STAT3 inhibition by either Stattic (STAT3-specific inhibitor) or STAT3-specific siRNA have improved the gefitinib efficacy, suggesting that disruption of PKM2-associated STAT3 could be a potential strategy to overcome EGFR-TKI resistance in CRC patients [129]. Moreover, *PKM2* expression is significantly associated with in vitro chemosensitivity to epirubicin (EPI) and 5-fluorouracil (5-fu) in breast cancer patients. Lin et al. showed a relationship between *PKM2* expression and patient's individual sensitivity to EPI and 5-fu, and proposed that patients with high PKM2 status should be considered for EPI based or EPI plus 5-fu chemotherapy to obtain better prognosis [130]. Briefly, PKM2 is clinically associated with chemoresistance to multiple anti-cancer drugs, and synergistic effects of combining PKM2 inhibitors with clinically used chemotherapeutic drugs could be more specific and promising in cancer therapies.

6.2. PKM2 activation

Tumor cells display a balance in metabolic flux to meet both the high energetic and biosynthetic demands of rapid growth and proliferation, which is regulated by various metabolic proteins and enzymes. One example is the phosphotyrosine-containg proteins that interact with PKM2 to promote its dimeric conformation by removing the allosteric activator FBP, thus redirect the glucose carbon flux into anabolic pathways to support cell proliferation [20]. However, small activator molecules can promote PKM2 tetramerization to limit the synthesis of cell building blocks and reduce its nuclear entry to attenuate gene transcription, suggesting that PKM2 activation can tactically inhibits cancer growth [131–133]. A natural compound 2′-hydroxycinnamaldehyde (HCA) activates tetrameric state of PKM2 and suppresses protein kinase activity by reducing the phosphorylation at T105, results in the inhibition of PKM2-mediated STAT3 phosphorylation at T705 [134]. Another small activator molecule has been identified that binds directly to the kinase pocket of PKM2 to reduce AKT phosphorylation and promote apoptosis in the non-small-cell lung cancer (NSCLC) cells in vitro [135]. Previously, two representative activator molecules (TEPP46 and DASA58) have been reported to enhance the association of PKM2 subunits into stable tetramers [136]. These findings suggest that PKM2 activators could be potential candidates in intervening cancer metabolism.

6.3. Polytherapeutic approaches

PKM2 inhibition or activation may not always be an effective mean to halt cancer growth, since the anti-tumor effects of PKM2 inhibitors or activators are likely dependent on cellular context, such as cell type and nutritional status. Therefore, the creation of new methods of targeting PKM2 or more broadly cancer metabolism should be a relevant problem at this stage. Currently, the main line of inquiry into PKM2-targeted cancer therapy is to exploit polytherapeutic strategies. For instance, hypoxia-resistant cancer cells are shown to overexpress PKM2 and glutaminase (GLS), and contrary to the separate treatments by siPKM2 and siGLS, their comingled effect was more substantial in tumor regression [137]. Moreover, there is evidence that many cancer cells overexpress glyoxalase 1 (GLO1), which is a ubiquitous enzyme involved in the detoxification of methylglyoxal, a highly cytotoxic by-product of glycolysis. The combined administration of shikonin and TLSC702 (GLO1 inhibitor) has significantly suppressed cell proliferation and induced apoptosis in tumor cells [138]. A greater combined effect of TEPP-46 and 2-deoxy-D-glucose (glycolysis inhibitor) has been shown in restraining tumor growth in vivo and in vitro [139]. Undoubtedly, these findings provide new directions for cancer treatment, and the concept of applying PKM2 inhibitors and activators

Box 2
Salient questions.

How PKM2 at various sub-cellular localizations coordinates complex molecular networks to meet the metabolic needs in tumor progression?

What cellular circumstances are critical for deciding the non-canonical localization of PKM2? How cellular sensors respond to stress conditions to promote the PKM2 translocation to mitochondria or extracellular space?

What sort of PTMs facilitate PKM2 packaging in microvesicles to accelerate the extracellular secretion?

In what oligomeric state (dimeric or monomeric) PKM2 exists in mitochondria and extracellular space? Whether the oligomeric regulation is dependent on the organelles specificity?

How important is the mitochondrial or extracellular localization of PKM2 for tumorigenesis? Which localization of PKM2 should be prioritized as a therapeutic target in cancer research and clinical trials?

Do PKM2 inhibitors or activators render solid tumors to sensitize chemotherapeutic drugs? Will the process cause toxic side effects?

simultaneously or employing them with other cellular contexts is evolving significantly.

7. Concluding remarks

Over the last two decades, PKM2 has been thoroughly studied in cancer cells concerning the gene expression, posttranslational modifications, allosteric regulation, expression shift of isoenzymes, non-canonical localization and numerous novel biological roles in cancer progression. Concentrating research efforts on understanding the moonlighting functions demonstrated that PKM2 has two facets; canonical and non-canonical. The canonical PKM2 is primarily exists in cytosol with metabolic function, whereas non-canonical PKM2 is localized to various subcellular localizations and fuels multiple aspects of cancer cells to sustain tumor growth. In addition, mounting evidence indicates that canonical and non-canonical PKM2 is notably involved in resistance acquisition processes against various chemotherapeutic drugs. Because of the moonlighting functions and pronounced role in drug resistance, PKM2 could be considered as a promising drug target in cancer therapy, however many unanswered questions are yet to be addressed (Box 1 & 2). Furthermore, PKM2 in other subcellular localization of tumor cells should be identified for improving the target identification in drug discovery process. It is believed that PKM2 could present a great potential for cancer diagnosis and treatment in the near future.

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Disclosure of conflict of interest

The authors show no conflict of interest.

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