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Review

The multifaceted regulation and functions of PKM2 in tumor progression

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ABSTRACT

Tumor cells undergo metabolic rewiring from oxidative phosphorylation towards aerobic glycolysis to maintain the increased anabolic requirements for cell proliferation. It is widely accepted that specific expression of the M2 type pyruvate kinase (PKM2) in tumor cells contributes to this aerobic glycolysis phenotype. To date, researchers have uncovered myriad forms of functional regulation for PKM2, which confers a growth advantage on the tumor cells to enable them to adapt to various microenvironmental signals. Here the richness of our understanding on the modulations and functions of PKM2 in tumor progression is reviewed, and some new insights into the paradoxical expression and functional differences of PKM2 in distinct cancer types are offered.

Contents

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1. Introduction

Pyruvate kinase (PK) catalyzes the final step reaction of glycolysis, converting phosphoenolpyruvate (PEP) and ADP to pyruvate and ATP. There are four isoforms of PK in mammals, termed PKL, PKR, PKM1 and PKM2. PKL and PKR are encoded by the same chromosomal gene PKLR and their expression is regulated by different promoters in specific tissues, with PKL expressed in the liver and kidneys and PKR expressed in red blood cells [1]. PKM1 and PKM2 are generated by the alternative splicing of two mutually exclusive exons in PKM gene. PKM1 is frequently highly expressed in adult tissue, while PKM2 is preferentially expressed in embryonic tissues and tumors [2].

Though PKM2 and PKM1 differ only by 22 amino acids, they are endowed with completely distinct regulatory properties. PKM1 forms a stable, constitutively active tetramer and has high pyruvate kinase activity, while PKM2 conformation is affected by numerous allosteric effectors and posttranslational modifications, and is switched between dimeric and tetrameric forms in tumor cells. Reversion from PKM1 to PKM2 is indispensable for shifting glucose metabolism towards aerobic glycolysis, a metabolic phenotype that is amenable to provide favorable energetics, biosynthetic intermediates and redox power for rapidly dividing tumor cells [3,4]. In addition, circulating, fecal or tissue levels of PKM2 are promising markers for diagnosis and prognosis of several types of cancer, especially the colorectal cancer [5–7].

It is now a popular truism that tumor transformation is paralleled by altered splicing of PKM pre-mRNA, switching the expression from PKM1 to PKM2. Nevertheless, recent studies indicate that this isoform switch pattern does not occur in all types of tumors, and PKM2 is also the predominant subtype over PKM1 in some non-transformed cells [8,9]. Furthermore, preferential expression of PKM2 is even reported to be dispensable for tumor progression in some types of tumors [10,11]. Another confusion that exists in literature concerning PKM2 is whether or not it can be exploited as a marker for screening or prognosis in cancers [12]. All of these research controversies may need more in-depth consideration. In this review, we will discuss the recent studies on the expression and activity of PKM2 with a major emphasis on its diverse functions and variable regulation.

2. Expression regulation of PKM2

2.1. Transcriptional regulation of PKM gene

The expression of PKM gene is driven by a plethora of signaling pathways that are affected by genetic mutations or tumor microenvironmental alterations. Several cellular signaling pathways, including hypoxia-inducible factor 1α (HIF-1α), nuclear factor kappa B (NF-κB), specificity protein 1 (Sp1), peroxisome proliferator-activated receptor gamma (PPARγ), *etc.*, have been identified to directly control PKM gene transcription (Fig. 1).

HIF-1α-mediated PKM transcription constitutes a positive feedback loop: HIF-1α binds to the hypoxia response element (HRE) within the first intron of PKM gene and stimulates PKM transcription. Intriguingly, PKM2 protein can interact with HIF-1α, further enhancing HIF-1α-mediated gene transcription, including PKM [13,14]. EGFR activation can stimulate NF-κBp65 binding to a putative NF-κB binding site in PKM gene promoter and activate PKM transcription. However, this process depends on the interaction of NF-kBp65 with HIF-1α, which acts as a coactivator for NF-κBp65 at target promoter regions [15] (Fig. 1).

Glucose stimulation can induce SP1 dephosphorylation *via* modulation of protein phosphatase 1, culminating in a higher SP1-dependent transcriptional activity, including PKM and aldolase [16]. In the PTEN-null mouse livers, activated Akt2 increases the expression of PPARγ, a multifunctional nuclear receptor, which directly binds to the PKM and hexokinase 2 gene promoters to activate the corresponding gene transcription, indicative of a contribution of the Akt2–PPARγ–PKM2 axis to liver malignancies [17,18] (Fig. 1).

Other aberrantly activated signaling pathways may act on these pervasive transcription factors, thereby indirectly driving PKM gene transcription in cancer cells. For example, mTOR signaling simultaneously activates HIF-1α-mediated PKM transcription and c-Myc-hnRNPs-dependent pre-mRNA splicing, resulting in the increased production of PKM2 mRNA [19]. Insulin can also activate the PI3K/mTOR/HIF-1α pathway to up-regulate PKM expression and modulate cancer cell metabolism [20]. Inactivation of Sprouty 2 (Spry2) tumor suppressor facilitates Akt-induced hepatocarcinogenesis through induction of MAPK activation and PKM2 expression. Spry2 inactivation-induced PKM2 expression is independent of MAPK, AKT/mTOR and c-Myc pathways, and thus the underlying molecular mechanisms need to be further identified [21].

2.2. Selective splicing of PKM gene

PKM1 and PKM2 mRNAs are generated by differential splicing of a common PKM pre-mRNA precursor, with PKM1 harboring exon 9 and PKM2 harboring exon 10. In proliferating and transformed cells, the oncogenic factor c-Myc is upregulated and activates the transcription of three heterogeneous nuclear ribonucleoproteins (hnRNPs), hnRNPA1, hnRNPA2 and hnRNPI, which bind repressively to sequences flanking exon 9, resulting in exon 10 inclusion and a higher PKM2/PKM1 ratio [22,23]. Interestingly, the outcome of PKM splicing can be determined by the concentration of these hnRNPs, and the spliced product containing both exon 9 and exon 10 will be degraded *via* the nonsense-mediated decay pathway [24]. In addition, generation of PKM2 mRNA can be achieved independent of the exon 9 repression by hnRNPs. SRSF3, a member of the serine/arginine-rich protein family of splicing factors, directly binds to the exonic splicing enhancer element in exon 10, resulting in the exon 10 inclusion and PKM2 mRNA production [25] (Fig. 1).

2.3. Epigenetic and microRNA regulation of PKM gene

The elevated PKM2 expression is well associated with the hypomethylation status in intron 1 of PKM gene in bladder, breast, colon and lung cancers, suggesting that epigenetic regulation of PKM expression by DNA methylation may be a pivotal mechanism for increased PKM2 expression in tumors [8]. PKM2 transcript can be targeted by a group of microRNAs at the posttranscriptional level, such as miR-326, miR-133a, miR-133b and miR-122, which can hamper PKM2 expression and affect cancer cell growth [26–28] (Fig. 1).

2.4. Stability regulation of PKM2 protein

The proteasomal and lysosomal pathways are the two main routes of protein and organelle clearance in eukaryotic cells [29]. The stability of PKM2 protein has been reported to be regulated by chaperone-mediated autophagy (CMA). High glucose concentration stimulates Lys305 acetylation of PKM2, which consequently increases its interaction with HSC70, a mediator for CMA pathway, and induces lysosome-dependent PKM2 degradation [30]. HSP40, a DNAJ motif containing chaperone, interacts with both PKM2 and HSC70, enhancing HSC70-mediated PKM2 degradation [31]. Proviral insertion in murine lymphomas 2 (PIM2), an oncogenic protein kinase, binds to PKM2 and raises its protein stability, an effect that is dependent on the Thr454 phosphorylation of PKM2 by PIM2 [32]. However, whether the stability regulation of PKM2 by PIM2 is associated with the proteasomal or lysosomal pathway remains to be unveiled. Together, PKM2 expression in cancer cells is rigorously controlled at epigenetic, transcriptional and posttranscriptional levels (Fig. 1).

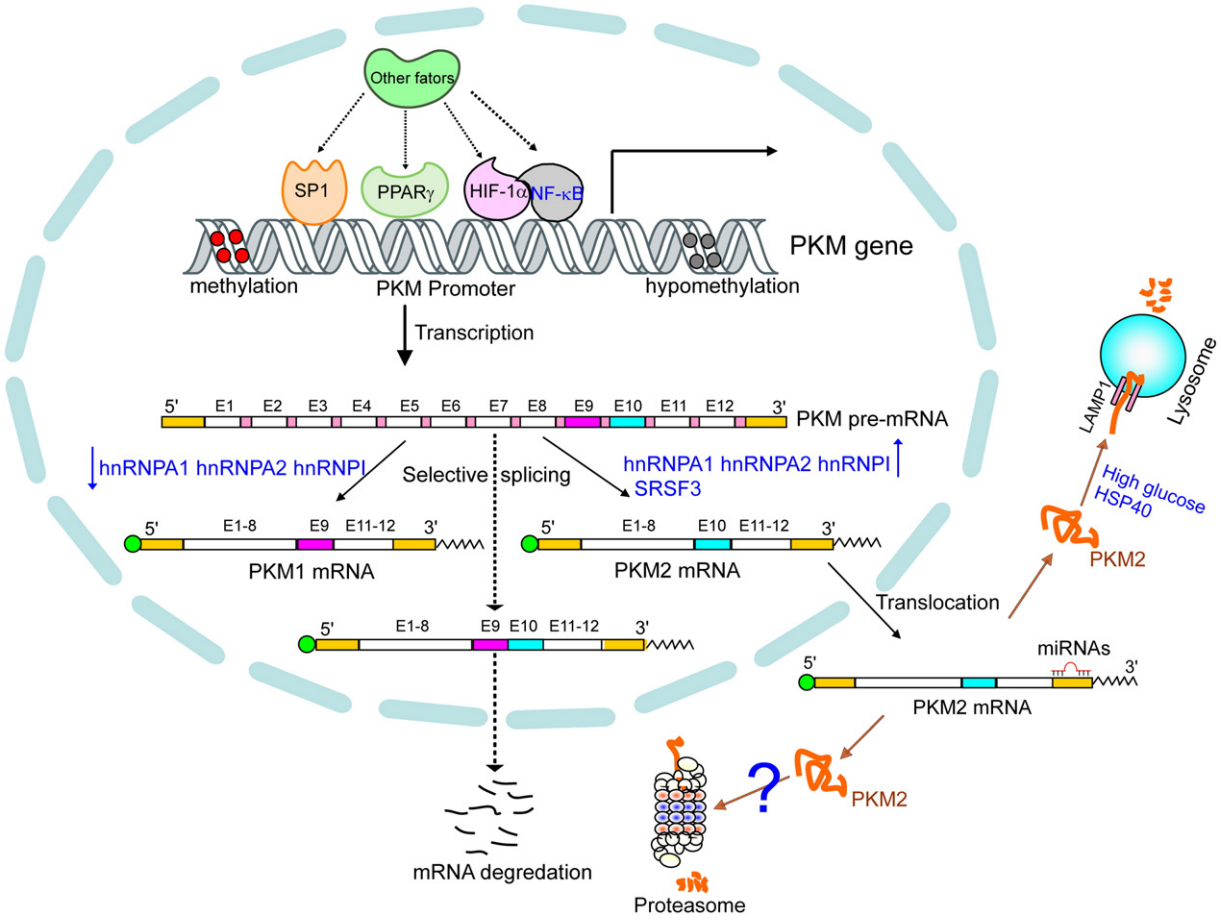

Fig. 1. Expression patterns of PKM2 in cancer cells. The expression of PKM2 is rigorously controlled at epigenetic, transcriptional and posttranscriptional levels. The intron 1 of PKM gene is hypomethylated in several types of cancer, which may be one of the causes for the high-PKM2 expression in cancer cells. PKM gene transcription is directly controlled by a plethora of transcriptional factors, including HIF-1α, NF-κB, Sp1 and PPARγ. The outcome of PKM gene splicing is determined by heterogeneous nuclear ribonucleoproteins hnRNPA1, hnRNPA2 and hnRNPI, or splicing factor SRSF3. High levels of hnRNP1/2/I or presence of SRSF3 leads to exon 10 inclusion but exon 9 exclusion, generating the PKM2 mRNA, and otherwise generating the PKM1 mRNA. The spliced product containing both exon 9 and exon 10 will be degraded *via* the nonsense-mediated decay pathway. PKM2 transcript can be targeted by a group of microRNAs, such as miR-326, miR-133a, miR-133b and miR-122, which can hamper PKM2 expression and affect cancer cell growth. The turnover of PKM2 protein is also regulated by the autophagy–lysosome pathway. Whether PKM2 stability is regulated by the ubiquitin–proteasome pathway is unknown.

2.5. New understandings of PKM2 expression pattern

It is well established that transformed tumor cells switch expression of PKM from normal tissue-expressed PKM1 to tumor-specific PKM2 *via* an alternative splicing mechanism [33]. Paradoxically, isoform switch from PKM1 to PKM2 is tissue-specific and only occurs in some types of tumors [8]. A recent study by Bluemlein et al. has also demonstrated that PKM2 is the predominant form over PKM1 in both transformed and non-transformed tissues, including kidney, lung, thyroid, bladder and colon, and that the PKM2 dominance in tumor is not ascribed to the switch form PKM1 to PKM2 isoform [9]. In addition, their finding do not support the current popular view that PKM2 is specifically expressed in proliferating cells, while PKM1 is mainly expressed in non-proliferating cells.

Even more surprisingly, many human tumors have a low or even negative PKM2 expression or activity, indicating that PKM2 expression is not consistently upregulated in human tumors. Large-scale sequencing has revealed several types of genetic mutations in PKM gene in multiple human cancer types, such as nonsense mutation and truncated mutation, which are expected to generate nonfunctional protein products or trigger nonsense-mediated decay of the transcripts [11,34]. Thus, the observed low expression or activity of PKM2 in some tumors may be due to the decreased transcription or translation, epigenetic silencing, or even the genetic mutation mentioned above. However, this simultaneously raises an inevitable question: how do cancer cells perform the metabolic program with only negligible pyruvate kinase expression or activity? One possible explanation may be that alternative pathways independently of pyruvate kinase may exist to allow the glycolytic flux to meet the cellular metabolic demands, which will be further discussed in Section 10 and Fig. 4.

Cancers may be driven by a reiterative process of somatic mutations and clonal selection, or directly derived from stem cells or progenitor cells [35,36]. Take the colorectal tumors for example, both “top-down” (colorectal adenomas grow across the mucosal surface and down into the crypts) and “bottom-up” theories have been proposed [37,38], indicating that colorectal cancer and other types of cancer may originate from committed cells or precursor stem cells [39–41]. In this case, two possibilities arise concerning the source of PKM2 in tumor cells: (1) If the tumor originates from the differentiated cells, the high levels of PKM2 in tumor cells are ascribed to the switch of PKM gene splicing from PKM1 to PKM2. (2) If the cell origins of tumors are precursor/stem cells, which already have a high level of PKM2, PKM2 expression in these tumor cells is likely directly inherited from the precursor/stem cells (Fig. 2).

3. Activity regulation of PKM2

3.1. Allosteric regulation of PKM2 by metabolic intermediates

In contrast to PKM1, which is present in a constitutively tetrameric active form, PKM2 undergoes conformational conversion between

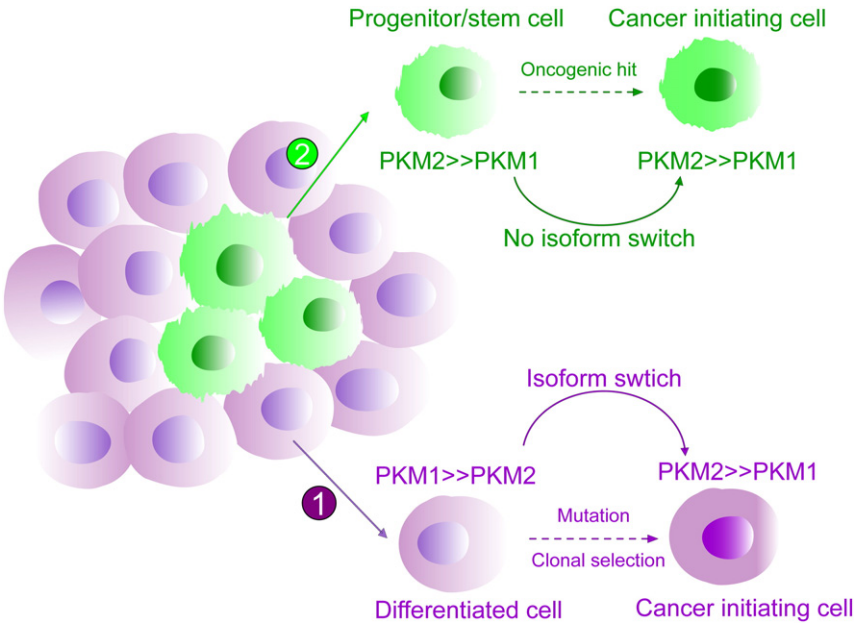

Fig. 2. Putative models of PKM1/2 isoform evolution during tumor formation. Cancers may be driven by a reiterative process of somatic mutations and clonal selection, or derived from stem cells or progenitor cells. Thus, there are at least two possibilities concerning the source of PKM2 in tumor cells: (1) If the tumor originates from the differentiated cells, the high levels of PKM2 in tumor cells are ascribed to the switch of PKM gene splicing from PKM1 to PKM2. (2) If the cell origins of tumors are precursor/stem cells, which already have a high level of PKM2 expression, PKM2 expression in these tumor cells is most likely directly succeeded from the precursor/stem cells.

tetramer and dimer, which respectively possesses pyruvate kinase activity and protein kinase activity [42]. Fructose-1,6-bisphosphate (FBP) and serine are both potent allosteric activators of PKM2, which directly bind to PKM2 and stabilize it in the active tetramer conformation [43–45]. Like PKM2, PKR and PKL can also be allosterically activated by FBP [46–48]. The concentration of succinyl-5-aminoimidazole-4-carboxamide-1-ribose-50-phosphate (SAICAR), an intermediate of the de novo purine nucleotide biosynthesis pathway, can be elevated upon glucose deficiency or EGFR activation. Surprisingly, binding of SAICAR to PKM2 can stimulate both the pyruvate kinase activity and the protein kinase activity of PKM2 [49,50]. This suggests that the SAICAR–PKM2 complex may utilize an identical active site for both protein and pyruvate kinase activities, using PEP as a common phosphate donor. However, the exact mechanism remains to be further elucidated. PKM2 is also allosterically inhibited by L-alanine, an effect that can be abrogated by FBP [51].

3.2. Activity regulation of PKM2 by posttranslational modification

The tetramer–dimer conversion of PKM2 is also modulated by posttranslational modification. Oncogenic tyrosine kinases, such as FGFR1, BCR-ABL, JAK2 and FLT3-ITD, are able to induce the phosphorylation of PKM2 at Tyr105, which culminates in the release of FBP from PKM2 and favors the dimer formation [52,53]. Tyr105 phosphorylation of PKM2 is negatively regulated by protein-tyrosine phosphatase 1B (PTP1B) in adipocytes, and reduced Tyr105 phosphorylation is associated with the development of glucose intolerance and insulin resistance in rodents, primates and humans [54]. Direct binding of phosphotyrosine peptides to PKM2 also catalyzes the release of FBP from PKM2 and impairs its pyruvate kinase activity [55]. Consequently, prolactin (PRL)-simulated PRL receptor phosphorylation impairs the enzymatic activity of PKM2 *via* direct interaction with PKM2 or recruitment of other proteins containing phosphorylated tyrosine residues [56].

In addition to being affected by the phosphorylated modification, PKM2 activity is also affected by a plethora of other types of posttranslational modification. High glucose can trigger PKM2 acetylation at Lys305, resulting in the decrease of pyruvate kinase activity and subsequent autophagic degradation of PKM2 [30]. Mitogenic and oncogenic stimuli induce p300-dependent Lys433 acetylation, which interferes with FBP binding and switch PKM2 from a cytoplasmic pyruvate kinase to a nuclear protein kinase [57]. In human lung cancer cells, acute increase of intracellular ROS causes pyruvate kinase activity inhibition through oxidation of Cys358 in PKM2 [58]. Interestingly, electrophilic modification of PKM2 at Lys367 by the glycolytic intermediate 1,3-bisphosphoglycerate also leads to pyruvate activity inhibition and allows glycolytic intermediates to enter the biosynthetic pathways [59]. In addition, O-methylated and sumoylation modification have also been identified in PKM2 protein, but whether these modifications are related to the activity and function of PKM2 remains to be clarified [60,61].

3.3. Activity regulation of PKM2 via protein–protein interaction

Several proteins are implicated in regulating PKM2 activity through direct protein–protein interaction. The C-terminal subunit of mucin1 (MUC1-C) binds to PKM2 at Cys474 and stimulates its pyruvate kinase activity, while the EGFR-phosphorylated MUC1-C interacts with PKM2 at Lys433 and inhibits its pyruvate kinase activity [62]. Death-associated protein kinase (DAPK), a multi-domain serine/threonine kinase, directly binds to PKM2 and functionally activates the pyruvate kinase activity [63]. PKM2 also interacts with the Jumonji C domain-containing dioxygenase (JMJD)5 and cytosolic promyelocytic leukemia (cPML), and both interactions result in the pyruvate kinase activity suppression [64,65]. Interestingly, the E7 oncoprotein from human papillomavirus (HPV) can also bind to PKM2 and shift its conformation from a tetrameric form to the dimeric state even in the presence of ample FBP [66]. Together, the tetramer–dimer conversion of PKM2 is a reversible process, and can be affected by metabolic intermediates, biochemical modification and tumor microenvironmental alterations, *etc.*, thereby accounting for the functional diversity of PKM2 in tumor

progression. The major functional sites affecting the conformational conversion or other properties of PKM2 have been listed in Table 1.

4. Metabolic regulation by PKM2

PKM2 isoform oscillates from high (tetrameric) to low (dimeric) pyruvate kinase activity. The dominance of dimeric form over tetrameric form provides a metabolic advantage to accumulate the metabolic intermediates upstream of pyruvate for the generation of nucleotides, proteins, and lipids required for cancer cell proliferation. Upon growth factor stimulation, PKM2 is phosphorylated at Tyr105 by oncogenic tyrosine kinases, or binds to the tyrosine-phosphorylated peptides, which compromises the pyruvate activity of PKM2 and shifts glucose metabolism from energy production towards anabolic processes [52,55,67,68]. In addition, the oncogenic serine/threonine kinase PIM2 interacts with PKM2, phosphorylating it at Thr-454 and thus promoting the aerobic glycolysis in cancer cells [69]. A high-glucose milieu stimulates acetylation of PKM2 at Lys305, which triggers the chaperone-mediated autophagic degradation of PKM2 and results in the subsequent accumulation of glycolytic intermediates required for tumor growth [30].

The pyruvate kinase activity of PKM2 is usually altered to accommodate for specific pathophysiological context [70,71]. Upon glucose deficiency, cellular SAICAR is increased in an oscillatory manner and stimulates the pyruvate kinase activity of PKM2 in cancer cells. The SAICAR–PKM2 interaction alters cellular energy level, glucose uptake and lactate production and ultimately promotes cancer cell survival in the nutrient-limited conditions [49]. SAICAR is also abundant in proliferating cells, especially upon epidermal growth factor receptor (EGFR) activation, and in these cases, PKM2–SAICAR acts as a protein kinase, which induces sustained ERK1/2 activation and is responsible for mitogen-induced cell proliferation [50]. PKM2 is also implicated in regulating intracellular reactive oxygen species (ROS) levels. An acute increase of ROS causes Cys358 oxidation of PKM2 and inhibits its pyruvate kinase activity, which increases glucose flux into the pentose phosphate pathway and thereby generates sufficient reducing potential for detoxification of ROS. Thus, Cys358 oxidation of PKM2 is instrumental to resist oxidative stress in cancer cells [58] (Fig. 3).

PKM2 plays a global regulatory role in biosynthetic amino acid metabolism and maintains cellular amino acid homeostasis, involving glutamine, serine and histidine, *etc.* [72]. Serine is a substrate for synthesis of biological macromolecules required for cell proliferation. It is also identified as an allosteric activator of PKM2, and controls PKM2 activity in a rheostat-like manner [73]. When serine is ample, PKM2 is fully active, enabling the maximal use of glucose *via* glycolysis. However, when serine is deficient, PKM2 activity is immediately reduced, enabling rapid diversion of glycolytic intermediates to serine biosynthesis and thus compensating for the serine shortfall. Serine deficiency coherently promotes the expression of enzymes required for serine synthesis from the accumulating glycolytic precursors [74] (Fig. 3). Interestingly, cells treated with small-molecule activators that bind to and lock PKM2 into the tetrameric conformation exhibit a profound dependency on serine for continued cell proliferation. This phenotype of serine auxotrophy is accompanied by increased expression of serine transporters and reduced carbon flux into serine biosynthetic pathway [75].

5. Signaling regulation by PKM2

Besides its established role in aerobic glycolysis, PKM2 also functions as a dimeric protein kinase. Mitogenic stimulation and oncogenic stimulation trigger Lys433 acetylation of PKM2 *via* p300 acetyltransferase. This modification prevents PKM2 activation by interfering with FBP binding and promotes its nuclear accumulation and protein kinase activity [57]. The nuclear translocated PKM2 dimer can use PEP as a phosphate donor, phosphorylating Stat3 at Tyr705, activating the MEK5 transcription and consequently accelerating cell proliferation and tumorigenesis [42] (Fig. 4).

Upon EGF stimulation, activated ERK2 stimulates Ser37 phosphorylation of PKM2, which then recruits PIN1 for cis–trans isomerization and subsequently binds to importin α5 for nuclear translocation.

Table 1
Main regulatory amino acid sites in PKM2 protein.

Active sites	Functions	Mutations	Refs.
Tyr105	Tyr105 phosphorylation disrupts PKM2 tetramer formation.	Loss-of-function: Y105F Gain-of-function: Y105E	[52,53]
Tyr105/148	Tyr105 and Tyr148 mediate the PTP1B–PKM2 interaction.	Loss-of-function: Y148F	[54]
Cys358	Cys358 oxidation by ROS causes pyruvate kinase activity inhibition.	Loss-of-function: (C358S)	[58]
Lys305	Lys305 acetylation results in a decrease of pyruvate kinase activity and autophagic degradation of PKM2.	Gain-of-function: K305Q	[30]
Pro403/408[a]	Pro 403/408 hydroxylation mediates the interaction of PKM2 with HIF-1α and promotes HIF-1α transactivation.	Loss-of-function: P403/408A	[13]
Lys433[a]	Lys 433 mediates the binding of phosphotyrosine peptides binding to PKM2. Lys 433 acetylation interferes with FBP binding and increases protein kinase activity.	Loss-of-function: K433E Gain-of-function: K433Q Loss-of-function: K433R	[55,57]
His464	His464 mediates the serine–PKM2 interaction.	Loss-of-function: H464A	[73]
Ser437	Ser437 is located in the FBP-binding pocket and involved in the hydrogen-bonding of FBP.	Loss-of-function: S437Y	[73]
Gln393[a]	Gln393 mediates the SAICAR–PKM2 interaction.	Loss-of-function: Q393K	[49]
Ser37	Ser 37 phosphorylation promotes PKM2 binding to importin α5 and subsequent nuclear translocation.	Loss-of-function: S37A Gain-of-function: S37D	[76]
Arg399[a] Arg400	Arg399/400 is essential for EGF-induced nuclear translocation of PKM2.	Loss-of-function: R399/400A	[76]
Arg399[a]	Arg399 plays a critical role in the formation of tetrameric PKM2.	Loss-of-function: R399E	[42]
Pro 408[a] Tyr 409[a]	Pro 408 and Tyr 409 mediate the binding of PKM2 with Bub3.	Loss-of-function: P408A Loss-of-function: T409A	[80]
Thr454	Phosphorylation of Thr454 by PIM2 results in an increase of PKM2 protein levels and aerobic glycolysis in cancer cells.	Loss-of-function: T454A	[69]
Lys 270	Lys 270 is directly involved in phosphoryl transfer from PEP.	Loss-of-function: K270M	[155]
His391 Lys422	Missense mutations of His391 (H391Y) and Lys422 (K422R) are present in Bloom syndrome, which stimulate more aggressive cancer metabolism in a dominant negative manner.		[34]

[a] These amino acids are specific to PKM2, while others are common to both PKM1 and PKM2.

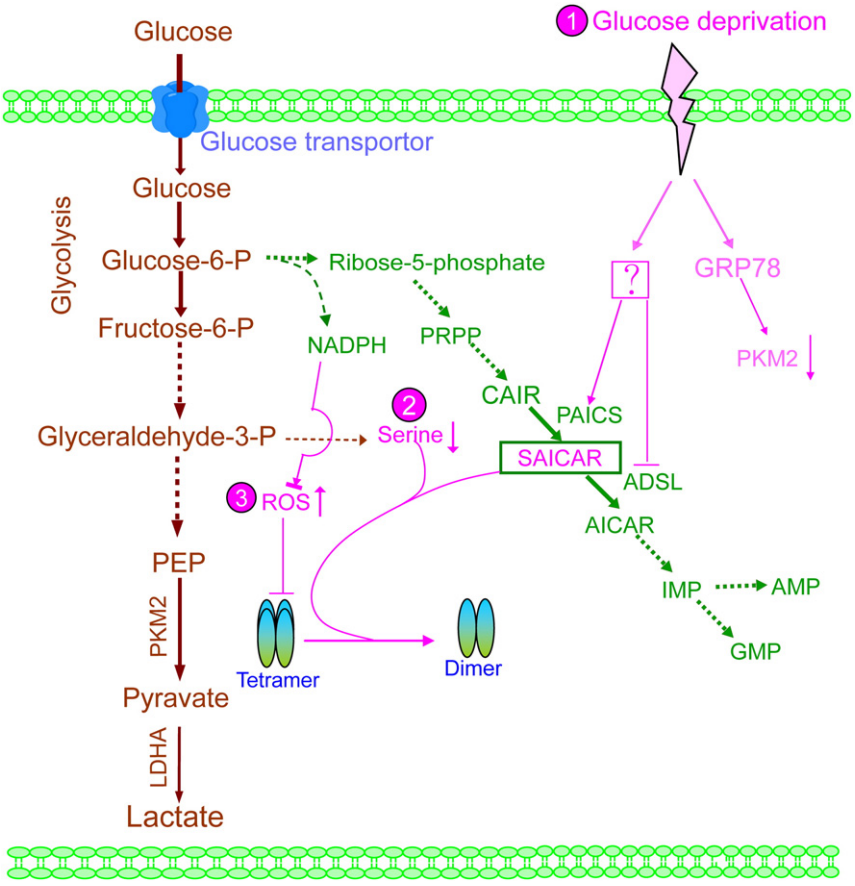

Fig. 3. Activity transition of PKM2 endows cancer cells with the plasticity to respond to different unfavorable conditions. In contrast to PKM1, PKM2 performs conformational conversion between tetramer (pyruvate kinase) and dimmer (protein kinase). The transformation/activity transition of PKM2 confers a survival advantage on cancer cells under the hostile conditions encountered within the microenvironment. For example, upon glucose deficiency, SAICAR is increased and SAICAR–PKM2 interaction stimulates the pyruvate kinase activity, resulting in the alteration of glucose metabolism and the ultimate cell survival. In addition, impaired PKM2 expression *via* induction of GRP78 is also conducive to cell survival. When serine is deficient, the pyruvate kinase activity of PKM2 is immediately reduced, enabling rapid diversion of glycolytic intermediates to serine biosynthesis and thus compensating for the serine shortfall, while acute ROS exposure causes Cys358 oxidation of PKM2, which inhibits the pyruvate kinase activity and increases glucose flux into the pentose phosphate pathway to generate sufficient reducing potential for detoxification of ROS.

Nuclear translocated PKM2 acts as a coactivator of β-catenin to induce cyclin D1 and c-Myc expression and the ensuing c-Myc-hnRNPI-dependent PKM2 expression [76,77]. Nuclear PKM2 protein kinase can induce Thr11 phosphorylation of histone H3 and the resultant dissociation of HDAC3 from the CCND1 and MYC promoter regions [78,79]. Strikingly, PKM2 also binds to and phosphorylates the spindle checkpoint protein Bub3 during mitosis, which is required for the recruitment of Bub3–Bub1 complex to kinetochores, and is also essential for the fidelity of chromosome segregation [80]. Together, PKM2-dependent transactivation of β-catenin and phosphorylation of histone H3 and Bub3 are instrumental for EGF-induced cell-cycle progression and brain tumorigenesis (Fig. 4).

Intratumoral hypoxia or mutation of von Hippel–Lindau (VHL) frequently activates HIF-1α [81]. Hypoxia also up-regulates the expression of JMJD5, a Jumonji C domain-containing dioxygenase, which directly interacts with PKM2 and disrupts its tetrameric pyruvate kinase activity. JMJD5 and PKM2, along with HIF-1α, are recruited the HREs, activating the transcription of HIF-1α target genes involved in glucose metabolism, including LDHA and PKM2 [64]. Similarly, Estradiol-17β induces the expression, splicing and nuclear translocation of PKM2 in human endometrial stromal cells, reprogramming glucose metabolism to support cell proliferation. PKM2 further interacts with Estradiol-17β receptor (ER)α and functions as an ERα co-activator to promote ERα transcriptional activity [82]. In addition, PKM2 interacts with Oct-4 through its C-terminal domain and enhances Oct-4-mediated transcription. This transactivating function of PKM2 may be implicated in regulating the pluripotent state of stem cells [83] (Fig. 4).

6. Roles of PKM2 in cell death regulation

Preferential expression of PKM2 in tumor cells is implicated in cell death and drug resistance. High expression of PKM2 elevates the level of anti-apoptotic protein Bcl-xL *via* enhancing NF-κBp65 stabilization, thereby promoting cell survival in gastric cancer cells [84]. Consistently, PKM2 knockdown increases caspase-3/7 activity and induces PARP cleavage, resulting in the decrease of clonogenicity in glioma cells [26]. *In vivo* delivery of siRNAs specifically targeting PKM2, but not PKM1, causes substantial tumor regression of established xenografts [85].

The roles of PKM2 in tumor cell apoptosis may be context- and cell type-dependent. At physiological concentrations of glucose, inhibition of PKM2 activity with a peptide aptamer hampers cell proliferation. Whereas under glucose-limited conditions, inhibition of PKM2 rescues cells from glucose deprivation-induced apoptotic cell death [86]. In addition, expression of PKM2 and its mediated glycolysis are also impaired by glucose deficiency *via* a GRP78-dependent signaling mechanism (Fig. 3, unpublished data). Surprisingly, in response to the structural somatostatin analog TT-232 and other apoptotic agents, such as H_2O_2 and UV irradiation, PKM2 translocates into the nucleus and performs a novel form of caspase- and Bcl-2-independent apoptosis, an event that is independent of its enzymatic activity [87].

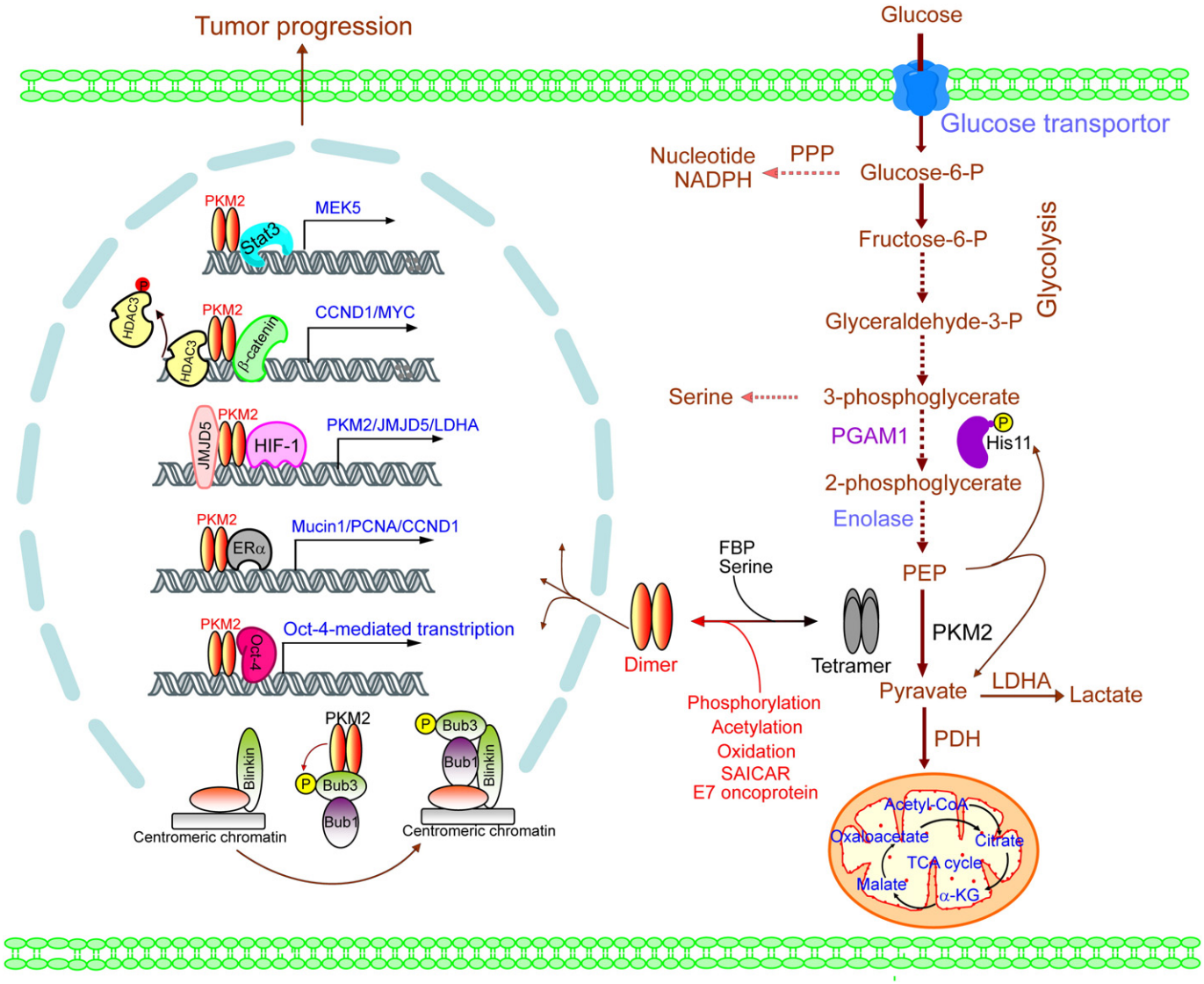

Fig. 4. Diverse functions of PKM2 in tumor progression. Though PKM2 is highly expressed, it is mainly present in the form of dimmer with low pyruvate kinase in cancer cells. In this case, the phosphate from PEP, the glycolytic substrate of PKM2, can be transferred to the catalytic histidine (His11) on phosphoglycerate mutase (PGAM1). This reaction occurs at physiological concentrations of PEP and produces pyruvate in the absence of pyruvate kinase activity. The dimeric PKM2 acts as a protein kinase or a coactivator for transcription factors, implicating in the activation of Stat3, β-catenin, HIF-1α, Oct-4 and ERα signaling pathways. In addition, PKM2 also binds to and phosphorylates the spindle checkpoint protein Bub3 during mitosis. The phosphorylated Bub3–Bub1 complex is then recruited to Blinkin on chromosomal kinetochores, which is required for the fidelity of chromosome segregation.

7. Roles of PKM2 in metastasis and angiogenesis

PKM2 has been reported to promote cell migration in colon and gastric carcinoma cells, and increases cell invasion in human biliary tract cancer [88–90]. We have recently found that PKM2 facilitates colon cancer cell adhesion and migration *via* a mechanism involving induction of Stat3 transcription, an effect that is dependent on its protein kinase activity rather than pyruvate kinase activity [91].

Angiogenesis is an important hallmark of tumor aggressiveness, and is an essential process required for tumor growth and metastasis [90]. Expression of PKM2 has been shown to be strongly correlated with that of VEGF, a potent angiogenic cytokine, in patients with advanced gastric cancer [92]. In addition, conditioned medium from PKM2-transfected cells significantly increases endothelial tube formation as revealed by the *in vitro* angiogenesis assay [90]. Though the detailed mechanism of how PKM2 is linked to angiogenesis remains unclear, the most likely scenario seems that PKM2 protein interacts with and transactivates HIF-1α, leading to an increased expression and secretion of HIF-1α target gene VEGF and thereby driving angiogenesis [13].

8. Roles of PKM2 in neoplastic transformation and cancer initiation

There is evidence indicating that PKM2 is implicated in tumor initiation and malignant transformation. Increased PKM2 expression accelerates 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced murine skin epidermal JB6 cell transformation. Thus PKM2 activation may be an early event of skin tumorigenesis and contributes to the malignant transformation of skin cells [93]. PKM2 is highly likely to be implicated in virus-induced cell transformation. Hepatitis C virus (HCV) RNA-dependent RNA polymerase NS5B interacts with PKM2. This interaction is required for HCV replication, thereby presumably contributing to HCV-associated hepatocellular carcinoma [96]. E7 oncoprotein, the human papillomavirus type 16 (HPV-16) gene product, binds to PKM2 and shifts its conformation from the tetrameric state to the dimeric

state. The interaction of E7 with PKM2 may promote the "aerobic glycolysis" phenotype and contribute to E7-induced cell transformation [66].

PKM2 expression is reduced during the process of embryonic stem cell differentiation, and the interaction of PKM2 and Oct-4 is most likely implicated in regulating the differentiation status of stem cells [83,94,95]. CD44, a cell surface marker for cancer stem cells, also interacts with PKM2, induces its tyrosine phosphorylation and enzymatic activity inhibition, and further enhances the glycolytic phenotype and metabolic flux to the pentose phosphate pathway. The metabolic modulation involving PKM2 and CD44 may contribute to the chemoresistant phenotype of cancer stem cells [96]. As mentioned above, PKM2 interacts with and transactivates β-catenin signaling, the activity of which is tightly intertwined with the phenotype of colon cancer stem cells [97], suggesting that expression of PKM2 may enhance colon cancer stemness by modulating β-catenin signaling.

9. Role of PKM2 in stromal–parenchymal interaction

Solid tumors can be recognized as organs composed of cancerous cells and non-cancerous cells, which mainly include cancer-associated fibroblasts (CAFs), myofibroblasts, endothelial cells, pericytes, mesenchymal stem cells and inflammatory cells [98–101]. Cancer cells trigger oxidative stress and autophagy in CAFs, leading to a loss of stromal caveolin-1 (Cav-1) and the ensuing up-regulation of both PKM1 and PKM2 [102]. The stromal expression of PKM1 and PKM2 drives tumor growth through different mechanisms: stromal PKM1 feeds tumor cells by increasing L-lactate secretion, while stromal PKM2 triggers a "pseudo-starvation" response and increases output of the ketone body 3-hydroxy-buryrate to fuel cancer cell mitochondrial respiration, thereby constituting a stromal–parenchymal metabolic coupling [103,104].

Dendritic cells (DCs) play a pivotal role in the induction of tumor-specific immune responses by enabling cross-priming where tumor antigens are presented by MHC I to $CD8^+$ cytotoxic T lymphocytes [105]. Several microenvironment factors, such as tumor-derived cytokines, hypoxia, and nutrient stress, are prone to affect the function and differentiation of DCs and contribute to their dysfunction [106,107]. Tumor-derived factors can up-regulate the expression of SOCS3, a member of the SOCS family proteins, which interacts with PKM2 at phosphorylated-Tyr105 and inhibits its pyruvate kinase activity in DCs. The interaction of SOCS3 with PKM2 results in decreased ATP production and DC dysfunction, and thus impairs DC-based immunotherapy against tumors [108].

10. New understandings of PKM2 function

Rapidly dividing cancer cells remodel their metabolism for efficiently incorporating nutrients such as glucose into biomass. PKM2 facilitates aerobic glycolysis and plays a key role in this metabolic rewiring. Paradoxically, the increased expression of PKM2 is accompanied by decreased pyruvate kinase activity, which is expected to restrict glycolysis. In this case, how does PKM2 perform aerobic glycolysis becomes enigmatic. In addition, there is evidence that PKM2 knockdown has no effects on established tumor xenograft growth, and that PKM2 loss even accelerates tumor formation in a mouse model of breast cancer [10,11]. These clues suggest that PKM2 mainly functions as a dimeric protein kinase, and its nonmetabolic functions are not absolutely necessary for tumor progression at least in some type of cancers. There should be other metabolic pathways bypass its function.

Interestingly, the phosphate from phosphoenolpyruvate (PEP), the glycolytic substrate of PKM2, can be transferred to the catalytic histidine (His11) on another glycolytic enzyme phosphoglycerate mutase (PGAM1) in PKM2-expressing cells. This reaction occurs at physiological concentrations of PEP and produces pyruvate in the absence of PKM2 activity. Thus, decreased pyruvate kinase activity in cancer cells allows PEP-dependent histidine phosphorylation of PGAM1 and provides an alternate glycolytic pathway that decouples ATP production from PEP-mediated phosphotransfer, allowing for the high rate of glycolysis to support the anabolic metabolism observed in many proliferating cells [109] (Fig. 4). Thus in cancer cells, oncogenic signalings or tumor microenvironmental alterations coordinate the conformation states of PKM2 through regulating the protein modifications or metabolite levels. Under the favorable conditions, PKM2 is most likely present in a dimer conformation, acting as a protein kinase to fuel tumor growth. In this case, an alternative glycolytic pathway may be activated to maintain the "aerobic glycolysis" phenotype. While under unfavorable conditions, PKM2 shifts to the tetrameric pyruvate kinase to sustain tumor cell survival.

11. Clinical implications

11.1. Screening, detection and prognosis

PKM2 is elevated in colorectal cancer patients as well as in inflammatory bowel disease (IBD) and is a sensitive and relatively specific marker for gastrointestinal pathology [110,111]. The determination of PKM2 in feces and plasma may provide a new promising screening tool for colorectal tumors, and has been recommended for early screening and detection of colorectal cancer [112–119]. In addition, plasma PKM2 may be used as a marker in pancreatic, gastric, esophageal, cervical cancer, cholangiocellular cancer, renal cell carcinoma and melanoma [120–123]. Circulating PKM2 also shows a prognostic value for disease recurrence in renal cell carcinoma after nephrectomy [124] and death risk of colorectal cancer [125]. However, other researchers have reported that PKM2 may be not a good screening marker for colorectal cancer [126–130], oral squamous cell carcinoma [131], renal cell carcinoma [132], Barrett's adenocarcinoma [133], and hematological malignancies [134]. Given some recent evidence that PKM2 is also abundant in at least some normal tissues [9], the potential of PKM2, no matter from the blood, tissues or feces, as a screening or prognostic marker for different types of cancer may require further investigation in much larger studies.

11.2. Therapeutic target

Targeting cancer metabolism has emerged as a hot topic for drug discovery. Given the high expression and important functions of PKM2 in modulating cancerous glucose metabolism, PKM2 may be exploited as a viable therapeutic target for some types of human malignancy [135]. There is evidence showing that knockdown of PKM2 by siRNA induces apoptosis in multiple cancer cell lines, and the delivery of PKM2 siRNA *in vivo* alone or combined with chemotherapy drugs causes substantial tumor regression of established xenografts [85,136]. The anti-tumor effects of cyclosporin A, resveratrol, shikonin, alkannin, vitamin K-3 and K-5 are also mediated by targeting PKM2 expression or activity [137–140]. Interestingly, oleanolic acid can induce isoform switch from PKM2 to PKM1 by virtue of blocking mTOR signaling and c-Myc-dependent hnRNPA1, consequently abrogating aerobic glycolysis in cancer cells [141]. Therefore, selective inhibition of PKM2 with small molecules may be feasible for developing anticancer agents [135,142].

Available evidence indicates that genetic replacement of PKM2 with the constitutively active PKM1 reduces the capacity of cancer cells to form tumors in nude mouse xenografts [3]. The fixation of PKM2 in the active tetrameric form by its activators is anticipated to decrease the glycolytic intermediates required for cell proliferation. A variety of PKM2 activators have been synthesized to activate its pyruvate kinase activity [143–148]. For example, synthetic PKM2 activator TEPP-46s can facilitate the assembly of PKM2 into stable tetramers, resulting in the increase of enzymatic activity to levels comparable to PKM1. Importantly, continuous dosing of mice with TEPP-46 decreases the progression of human cancer cell xenografts [149]. However, inhibition or activation of PKM2 may not always provide an efficacious treatment for cancer [10], and the anti-tumor effects of PKM2 inhibitors or activators are likely dependent on the cellular context, such as cell type,

nutritional status [150–152]. Thus identifying the conditions whereby inhibition or activation of PKM2 would hamper cancer cell growth is instrumental in cancer therapy targeting PKM2.

12. Concluding remarks

Metabolic pathways are providing a large hunting ground to discover potential therapeutic targets in malignancy. A large body of evidence indicates that cancer cells undergo metabolic alterations for their persistent growth and become particularly addicted to the rapacious uptake of glucose, glutamine [153] or dependent on other types of nutrients [154]. Despite the multifaceted metabolic and non-metabolic functions of PKM2 in cancer progression, a central question, as yet unaddressed, is whether the preferential expression and pyruvate/protein kinase activity transition of PKM2 are indispensable for the growth and survival of most, if not all, types of cancer.

Additionally, several other important questions still remain regarding the feasibility of using PKM2 as a drug target. First, is the function of PKM2 related to specific genetic context or microenvironmental milieu? Second, whether other pathways will be activated to compensate for the defective PKM2 function caused by human intervention? If so, adding combinations of inhibitors directed against the compensatory events may be more effective.

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