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Emerging roles of PKM2 in cell metabolism and cancer progression

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Abstract

Increased conversion of glucose to lactate is a key feature of many cancer cells that promotes rapid growth. Pyruvate kinase M2 (PKM2) expression is increased and facilitates lactate production in cancer cells. Modulation of PKM2 catalytic activity also regulates synthesis of DNA and lipids required for cell proliferation and NADPH required for redox homeostasis. In addition to its role as a pyruvate kinase, PKM2 also functions as a protein kinase and as a transcriptional coactivator. These biochemical activities are controlled by allosteric regulators and post-translational modifications of PKM2 that include acetylation, oxidation, phosphorylation, prolyl hydroxylation, and sumoylation. Given its pleiotropic effects on cancer biology, PKM2 represents an attractive target for cancer therapy.

Altered glucose metabolism in cancer cells

Most differentiated cells utilize glucose as one of the major sources of energy for their growth and survival [1]. Under well-oxygenated conditions, glucose is completely burned to CO_2 and H_2O via glycolysis and the tricarboxylic acid (TCA) cycle, and through the process of oxidative phosphorylation generates the high-energy compound ATP. When cellular O_2

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availability becomes limited, glucose is predominantly metabolized to lactate, which generates only 2 moles of ATP per mole of glucose. Although the conversion of glucose to lactate is much less energy efficient, it is a major pathway of glucose metabolism in cancer cells even in the presence of O_2, a phenomenon that was discovered by Otto Warburg in 1924 and has become known as the Warburg effect or aerobic glycolysis [2]. V-SRC was the first oncoprotein shown to stimulate aerobic glycolysis [3-5]. The cofactors NADH and NAD^+ are also the important determinants of lactate production [6]. The Warburg effect is associated with a high rate of glucose uptake relative to O_2 consumption. Glucose is also utilized by cancer cells to generate macromolecular building blocks (nucleotides, amino acids, and acetyl CoA) that are required for cell proliferation [7]. Understanding of the Warburg effect is complicated by the fact that advanced cancers are characterized by intratumoral hypoxia, which also stimulates lactate production. Lactate secreted by hypoxic cells can serve as an energy source for well-oxygenated cells [8].

Hypoxia-inducible factor 1 (HIF-1) is a master regulator of adaptive responses to reduced O_2 availability, and HIF-1 is activated by intratumoral hypoxia and/or genetic alterations in the majority of advanced human cancers [9]. HIF-1 is a heterodimeric transcription factor, consisting of an O_2-regulated HIF-1α subunit and a constitutively expressed HIF-1β subunit [10]. In well-oxygenated cells, HIF-1α is hydroxylated at proline 402 and 564 by the prolyl hydroxylase domain proteins, PHD1-3, which utilize O_2 and α-ketoglutarate as substrates [11]. Prolyl-hydroxylated HIF-1α is bound by the von Hippel-Lindau (VHL) tumor suppressor protein, which is the substrate recognition component of an E3 ubiquitin ligase that targets HIF-1α for proteasomal degradation [12]. Under hypoxic conditions, HIF-1 activates the transcription of genes encoding glucose transporters and glycolytic enzymes, thereby enhancing glucose uptake and glycolytic flux (Figure 1) [13]. HIF-1 also controls the expression of lactate dehydrogenase A (LDHA) and pyruvate dehydrogenase kinase 1 (PDK1) (Figure 1) [13]. As a result, HIF-1 activation shifts the cell from oxidative to glycolytic metabolism and mediates the Warburg effect in VHL-null renal carcinoma cells [14]. The oncoprotein MYC also regulates glucose metabolism through activating transcription of metabolic genes including *HK2*, *PKM2*, *LDHA*, and *PDK1* [15]. When ectopically expressed, MYC cooperates with HIF-1 to stimulate expression of PDK1 and HK2 [16]. Thus, HIF-1 and MYC both play critical roles in promoting the Warburg effect.

Although Otto Warburg first observed altered glucose metabolism in cancers ninety years ago, novel mechanisms underlying the Warburg effect in cancer cells continue to be elucidated. Recent studies of the M2 isoform of the glycolytic enzyme pyruvate kinase (PKM2) illustrate the complex nature of the Warburg effect in cancer cells. Expression of PKM2 is increased among diverse human cancers in lung, breast, prostate, blood, cervix, kidney, bladder, and colon, compared to the matched normal tissues [17]. Several novel roles for PKM2 in human cancers have emerged from recent studies and we will review the current understanding of molecular mechanisms by which PKM2 exerts effects on cellular metabolism, redox homeostasis, proliferation and other aspects of cancer biology. We will also discuss the implications of these findings with respect to potential therapeutic targeting of PKM2.

Regulation of PKM2 expression in cancer cells

Pyruvate kinase, a rate-limiting glycolytic enzyme that catalyzes the conversion of phosphoenopyruvate (PEP) and ADP to pyruvate and ATP, consists of four isoforms in mammals: PKL, which is expressed in liver and kidney; PKR, which is expressed in erythrocytes; PKM1, which is predominantly expressed in adult muscle, brain, bladder, and fibroblasts; and PKM2, which is expressed in most cells except for adult muscle cells (Figure 2) [17-19]. PKL, PKR, and PKM1 exist as stable tetramers, whereas PKM2 subunits

form tetramers and dimers [18]. The dimeric PKM2 has higher K_m value for the substrate PEP than the tetrameric PKM2, and thus the dimeric form is inactive at physiological concentrations of PEP [18, 20].

PKM1 and PKM2 differ at 23 amino acids residues and both are encoded by the *PKM2* gene through alternative splicing of the same primary RNA transcript (Figure 2): PKM1 mRNA contains exon 9 and lacks exon 10-encoded sequences, whereas PKM2 contains exon 10 and lacks exon 9-encoded sequences [21-24]. Heterogeneous nuclear ribonucleoproteins (hnRNPs) I (also known as PTB), hnRNPA1, and hnRNPA2 bind to intronic sequences flanking exon 9 of PKM2 RNA and promote exon 9 excision during processing of the primary RNA in cancer cells [22, 23]. In cells expressing low levels of hnRNPI, hnRNPA1, and hnRNPA2, splicing repressors bind to intronic sequences flanking exon 10 and promote processing to PKM1 mRNA [24]. On the other hand, SRSF3 binds to a splicing enhancer element in the exon 10-encoded RNA sequence to promote processing to PKM2 mRNA [25]. Knockdown of SRSF3 increases PKM1 expression in transformed HEK293 cells [25]. PKM2 mRNA species that contain both exon 9 and exon 10 are degraded by nonsense-mediated decay [24].

PKM2 gene transcription is controlled by several factors. Sp1 constitutively activates transcription of the *PKM2* gene, whereas Sp3 functions as a transcriptional repressor that dissociates from the *PKM2* gene under hypoxic conditions [26]. HIF-1 activates *PKM2* gene transcription in hypoxic cells by binding to a hypoxia response element (HRE) located within the first intron of the *PKM2* gene [27]. *PKM2* expression is also regulated by MYC both directly, by binding to a MYC response element located in the promoter of the *PKM2* gene [15], and indirectly, by activating transcription of the genes encoding hnRNPI, hnRNPA1, and hnRNPA2, which facilitate the alternative splicing that generates PKM2 mRNA [22]. Mammalian target of rapamycin (mTOR), a serine/threonine protein kinase that regulates cell growth, survival, and protein synthesis, indirectly stimulates PKM2 expression through activation of HIF-1 and MYC [28]. Another serine/threonine protein kinase, Akt2, promotes PKM2 expression in the liver of *PTEN*-deficient mice through increased expression of peroxisome proliferator-activated receptor γ, which activates *PKM2* gene transcription [29]. PKM2 expression is reduced in mouse embryonic fibroblasts isolated from PTEN-overexpressing transgenic mice [30].

MicroRNAs (miRs) also regulate the synthesis of PKM2 protein. miR-326 targets two regions in the 3′-untranslated region of PKM2 mRNA and transfection of glioma cells with a miR-326 precursor decreased PKM2 protein levels and inhibited translation of a chimeric mRNA consisting of luciferase coding sequences upstream of PKM2 3′-untranslated sequences that included the putative miR-326 binding sites [31]. miR-133a and miR-133b also inhibited PKM2 expression in cancer cells [32]. PKM2 overexpression is correlated with downregulation of miR-133a and miR-133b. The mechanism for this mutual antagonism between miR-133a/b and PKM2 has not been determined.

PKM2 is allosterically activated by the glycolytic metabolite fructose-1,6-biphosphate (FBP) [33, 34]. The crystal structure shows that FBP binds tightly to tetrameric PKM2 [34]. The release of FBP causes PKM2 to switch from the tetrameric to dimeric form and decreases its catalytic activity [18, 33]. The dimeric form of PKM2 has low affinity for PEP, leading to the accumulation of glycolytic metabolites, which can be utilized for anabolic metabolism via the pentose phosphate pathway (PPP) [35]. Tyrosine-phosphorylated peptides bind to PKM2, release FBP, stabilize the dimeric form, decrease PKM2 activity, and promote cancer cell proliferation [33]. PKM2 itself was phosphorylated at tyrosine residues 83, 105, 148, 175, 370, and 390 by fibroblast growth factor receptor type 1 (FGFR1) (Table 1) [36]. Phosphorylation of PKM2 at tyrosine 105 induced FBP release

from active tetrameric PKM2, promoted dimer formation, and decreased PKM2 enzymatic activity. In contrast, the phosphorylation of PKM2 at other tyrosine residues by FGFR1 failed to regulate PKM2 activity.

Serine also functions as an allosteric activator for PKM2 [37]. Serine at millimolar concentrations increased PKM2 enzymatic activity in the absence of FBP. PKM1 expression switch to PKM2 resulted in the accumulation of PEP and 3-phosphoglycerate and stimulated serine synthesis in non-small-cell lung carcinoma cells (Figure 1) [37].

In addition to allosteric regulators, PKM2 activity is also regulated by post-translational modifications. Acetylation of PKM2 at lysine 305 led to decreased PKM2 activity (Table 1) and the accumulation of glycolytic metabolites such as glucose-6-phosphate, fructose-6-phosphate, and FBP, whereas pyruvate and lactate levels were decreased [38]. The acetyltransferase PCAF catalyzed lysine acetylation of PKM2 and treatment of cells with histone deacetylase inhibitors trichostatin A and nicotinamide led to increased PKM2 acetylation [38]. PKM2 acetylation was also increased in cells cultured under high concentrations of glucose [38]. PKM2 protein levels were decreased in cells exposed to high concentrations of glucose due to Hsc70-dependent chaperone-mediated autophagy (CMA) [38]. Hsc70 preferentially bound to acetylated PKM2 and diverted it to the lysosome for degradation. Knockdown of Hsc70 or the lysosomal membrane protein LAMP2A led to the accumulation of PKM2 protein by blocking high glucose-induced, CMA-dependent PKM2 degradation. The lysosomal protease inhibitor leupeptin increased PKM2 protein levels, whereas 6-aminonicotinamide, an activator of CMA, decreased PKM2 levels [38].

PKM2 activity is also modulated by interacting proteins. Mucin 1 and death-associated protein kinase bind to PKM2 and increase its catalytic activity, which leads to enhanced glycolysis in cancer cells [39, 40]. In contrast, the promyelocytic leukemia (PML) tumor suppressor protein reduces PKM2 activity [41].

PKM2 promotes the Warburg effect in cancer cells

PKM2 levels are increased in human cancer biopsies, compared to the adjacent normal tissues [17]. PKM2 expression is associated with increased glucose uptake, increased lactate production, and decreased O_2 consumption, which can be reversed by genetic manipulations that replace PKM2 expression with PKM1 [42]. Therefore, PKM2, but not PKM1, promotes the Warburg effect. Cancer cells expressing PKM2 grow more rapidly in tissue culture and as xenografts in mice, compared to cells expressing PKM1 [42]. PKM2 can be switched between dimer and tetramer in cancer cells [18]. It is therefore speculated that, when PKM2 exists as dimer in cancer cells, high expression of PKM2 leads to anabolic metabolism of glucose for macromolecular biosynthesis rather than oxidative metabolism for energy production (Figure 1), which benefits cancer cell proliferation and tumor growth.

The alterations in PKM2 expression and activity discussed above could not account for increased lactate production and reduced oxidative phosphorylation in cancer cells expressing PKM2, because PKM2 catalyzes the conversion of PEP to pyruvate, which can be metabolized either to lactate (by LDHA) or to acetyl CoA (by pyruvate dehydrogenase); i.e. PKM2 is upstream of the decision point between oxidative and glycolytic metabolism. Furthermore, as described above, the reduced catalytic activity of PKM2 is believed to reduce lactate production by shunting glycolytic metabolites to the PPP in cancer cells. A molecular mechanism by which PKM2 may mediate the Warburg effect in cancer cells has been delineated (Figure 1) [27, 43]. PKM2 interacts with HIF-1α in the nucleus and functions as a transcriptional co-activator for HIF-1 to stimulate the expression of HIF-1 target genes including *SLC2A1* (which encodes glucose transporter 1), *LDHA*, and *PDK1* in HeLa cervical carcinoma and Hep3B hepatoblastoma cells (Table 2) [27]. PKM2 co-

activator function is independent of its catalytic activity as a glycolytic enzyme [27]. PKM2-stimulated expression of HIF-1 target genes promotes the shift from oxidative phosphorylation to glycolytic metabolism. However, PKM1 failed to activate HIF-1 in cancer cells, which may explain why PKM1 cannot mediate the Warburg effect [42]. PKM2 also binds to HIF-2α and promotes HIF-2-mediated transactivation in cancer cells [27]. In addition to its effects on transcription of metabolic genes, PKM2 stimulates HIF-1- and HIF-2-mediated expression of the *VEGFA* gene (which encodes vascular endothelial growth factor), thereby promoting angiogenesis (Table 2) [27]. Thus, PKM2 plays a broader role in promoting cancer progression than was previously appreciated [43].

Epigenetic modification of the core histone H3 was associated with the HIF-1/PKM2-mediated Warburg effect in cancer cells [27]. PKM2 interacted with the histone acetyl transferase p300 and increased the recruitment of p300 to the HREs of HIF-1 target genes to induce acetylation of histone H3 at lysine 9. PKM2 enhanced HIF-1 binding to the HREs and stimulated HIF-1-mediated transactivation.

A conserved prolyl hydroxylation motif (LXXLAP, where X is any amino acid) was identified in the exon 10-encoded domain that is uniquely present in PKM2 [27]. Mass spectrometry revealed that PKM2 was hydroxylated on proline residues 403 and 408, which was catalyzed by PHD3 (Table 1). Prolyl hydroxylation facilitated PKM2 binding to HIF-1α to enhance HIF-1-mediated transactivation in VHL-null RCC4 renal carcinoma cells, which provides a molecular basis for the selective effect of PKM2 in stimulating the Warburg effect in kidney cancer cells [27, 43].

PKM2 and PHD3 are both encoded by HIF-1 target genes [27, 44]. Increased expression of PHD3 is induced by HIF-1 under hypoxic conditions, which compensates for reduced hydroxylase activity, and induces prolyl hydroxylation of PKM2, thereby promoting transcription of HIF-1 target genes [27]. Thus, PHD3 and PKM2 exert a positive feedback loop in cancer cells that amplifies HIF-1 activity, which may play a major role in driving metabolic reprogramming and other aspects of cancer biology that are mediated by HIF-1 [45].

Other effects of PKM2 on gene transcription in cancer cells

PKM2 has also been shown to translocate into the nucleus in response to interleukin-3 or apoptotic agents such as the somatostatin analogue TT-232, hydrogen peroxide, or ultraviolet irradiation [46, 47]. A recent study demonstrated that epidermal growth factor (EGF) also induced PKM2 translocation into the nucleus of multiple cancer cells, including U87 glioblastoma cells, DU145 prostate cancer cells, and MDA-MB-231 breast cancer cells [48]. Nuclear PKM2 stimulated EGF-induced, β-catenin-mediated transactivation in U87 cells (Figure 1). Activation of the EGF receptor (EGFR) by EGF binding stimulated c-Src kinase, which phosphorylated β-catenin at tyrosine 333 [48]. In line with the finding that PKM2 is a phosphotyrosine-binding protein [33], PKM2 predominantly bound to tyrosine phosphorylated β-catenin in the nucleus to occupy the promoter of the β-catenin target gene *CCND1*, thereby dissociating HDAC3 from the *CCND1* promoter and enhancing acetylation of histone H3 at the *CCND1* gene to stimulate cyclin D1 expression (Table 2) [48]. Although PKM1 is also expressed in glioblastoma [22], it may not regulate β-catenin transactivation because PKM1 is not able to bind to tyrosine phosphorylated proteins such as β-catenin [33]. A catalytically inactive PKM2 (K367M) mutant failed to induce EGF-mediated cyclin D1 expression because it did not stimulate HDAC3 dissociation from the *CCND1* gene upon EGFR activation, although the mutant PKM2 protein translocated into the nucleus and bound to the *CCND1* gene [48]. These findings demonstrated a novel role for PKM2 catalytic activity in regulating gene transcription in the nucleus, in addition to its

role as a glycolytic enzyme in the cytosol. However, the substrate for phosphorylation by PKM2 in this context has not been identified. PKM2 knockdown also inhibited EGF-enhanced expression of the β-catenin target gene *MYC* (Table 2) [48]. As described above, MYC stimulates *PKM2* transcription and mRNA splicing in cancer cells [22]. Thus, PKM2 and MYC are involved in a feed-forward mechanism similar to that described above for HIF-1 and PKM2 (Figure 3). The PKM2-β-catenin interaction promoted cancer cell proliferation and tumor growth in xenograft mice, but it remains unclear whether β-catenin regulates (via MYC) the PKM2-mediated Warburg effect in cancer cells.

A recent study demonstrated that nuclear PKM2 existed in dimeric form, whereas cytosolic PKM2 appeared to include both dimeric and tetrameric forms [49]. Interestingly, dimeric PKM2 acted as a protein kinase, whereas tetrameric PKM2 functioned as an active pyruvate kinase. The protein kinase activity may be specific for PKM2, but not for PKM1, because PKM1 exists as tetramer [18]. Nuclear PKM2 phosphorylated the transcription factor STAT3 using PEP as a phosphate donor and activation of STAT3 by PKM2 stimulated transcription of the *MEK5* gene to increase cell proliferation (Figure 1) [49]. Mutation of arginine-399 to glutamate (R399E) disrupted PKM2 tetramer formation. Expression of the dimeric PKM2 (R399E) mutant increased phosphorylation of STAT3 *in vitro* and in cells, and also promoted cancer cell proliferation and tumor growth [49]. Future studies are required to determine whether PKM2 phosphorylates other target proteins in cancer cells, which may reveal additional novel functions of PKM2.

In addition to transcription of genes encoding proteins that are required for cancer metabolism and cell proliferation, PKM2 regulates stem cell reprogramming through co-activating the transcription factor Oct-4 [50]. PKM2 bound to the DNA binding domain of Oct-4 and stimulated Oct-4-mediated transactivation (Figure 1). However, the Oct-4 downstream genes regulated by PKM2 remain to be determined.

PKM2 and oxidative stress in cancer cells

Reactive oxygen species (ROS) are produced from O_2 mainly through mitochondrial respiration and the activities of NAPDH oxidase, xanthine oxidase, and 5-lipoxygenase [51]. Many cancer cells generate more ROS than non-transformed cells in response to stimuli such as phorbol esters and hypoxia/reoxygenation [52, 53]. ROS has been implicated in DNA damage, cell proliferation, cell survival, and cell mobility, which promote tumor initiation and progression [53]. Increased ROS also induces oxidation of proteins and lipids in cells. PKM2 was shown to be oxidized in lung cancer cells exposed to oxidative stresses such as hydrogen peroxide, diamide and hypoxia [54]. Structural and biochemical analyses demonstrated that cysteine 358 in PKM2 was oxidized in cells treated with diamide (Table 1). Cysteine oxidation impaired PKM2 subunit association and decreased PKM2 activity, leading to the accumulation of glucose-6-phosphate and increased glucose flux through the PPP in A549 cells [54]. The PPP is a critical source of reduced NADPH and activation of the PPP by PKM2 oxidation reduced intracellular ROS and promoted cell survival and tumor growth during oxidative stress (Figure 1) [54]. It has been shown that the PPP regulates gene transcription during the antioxidant response [55]. However, it remains unknown whether activation of the PPP by PKM2 oxidation regulates to PKM2-mediated gene transcription in cancer cells.

A similar effect of PKM2 on antioxidant responses was also demonstrated in respiring yeast [35]. In yeast, less active PKM2 accumulated PEP, which in turn provides a feedback loop inhibiting the upstream glycolytic enzyme triosephosphate isomerase (TPI) to stimulate the PPP. The PKM2-PEP-TPI feedback loop prevented ROS accumulation during respiration in

yeast. This mechanism for maintenance of redox balance may exist in human cells as well because human TPI was also efficiently inhibited by PEP [35].

PKM2: a potential target for cancer therapy

Targeting cancer metabolism is considered an attractive strategy for cancer therapy. A high-throughput screen identified small molecules that targeted the allosteric regulation of PKM2 catalytic activity [56]. Compound 3, the most effective PKM2 inhibitor identified in the screen, inhibited PKM2 activity at micromolar concentrations in H1299 cells, which could not be reversed by the addition of FBP to cell lysates [56]. Compound 3 was toxic to cells and increased cell death under different physiological and pathological conditions. Although Compound 3 had little inhibitory effect on PKM1, it also inhibited the allosteric activity of PKL and PKR. The enantiomeric naphthoquinone shikonin and its analog alkannin also inhibited PKM2 activity in cancer cells [57]. The inhibition of PKM2 activity by shikonin and alkannin was much greater than compound 3. Low concentrations of shikonin and alkannin selectively targeted PKM2, whereas high concentrations also inhibited PKM1 and PKL [57]. Therefore, it remains a challenge to identify inhibitors that specifically target PKM2 because there is a high similarity of protein structure and biochemical properties among the four isoforms of pyruvate kinase.

Small molecule PKM2 activators were also identified in a high-throughput screen [58]. The rationale of PKM2 activators for cancer therapy is that increasing PKM2 activity promotes lactate production and reduces the accumulation of glycolytic intermediates for anabolic metabolism that is critical for cancer cell proliferation. The PKM2 activator DASA-10 prevented ROS-induced decrease in PKM2 activity and glucose-6-phosphate accumulation in A549 cells [54]. However, PKM2 inhibitors and activators may not modulate non-catalytic effects of PKM2 in human cancers, such as the PKM2-dependent regulation of transcription of genes that are involved in metabolic reprogramming and angiogenesis (Table 2), which are independent of its enzymatic activity [27, 50]. Additional studies are required to determine the effects (if any) of PKM2 inhibitors and activators on PKM2-mediated gene expression.

Small interfering RNA (siRNA) specifically targeting PKM2 mRNA has been shown to inhibit tumor growth in xenograft mice. PKM2 siRNA-156 induced caspase-3/7-mediated apoptosis of diverse cancer cells, but had no detrimental effects on the survival of normal adult skin fibroblasts or umbilical vein endothelial cells [59]. Intratumoral injection of PKM2 siRNA-156 reduced tumor growth in mice [59]. Inhibition of PKM2 expression by short hairpin RNA also increased the efficacy of anticancer drugs in mice [60, 61]. HIF-1 has been shown to control the transcription of drug resistance genes and thereby mediate resistance to cancer chemotherapy [62], suggesting a potential mechanism underlying these observations.

Concluding remarks

Increased expression of PKM2 leads to increased glucose uptake, accumulation of glycolytic metabolites, and metabolic reprogramming in cancer cells, which provides advantages for cancer cell growth and survival. PKM2 also prevents intracellular ROS accumulation, thereby promoting cancer cell survival under oxidative stress. Emerging roles of PKM2 in gene transcription have offered novel insights into PKM2-mediated cancer progression. Future genome-wide studies to identify PKM2 target genes would improve our understanding of the changes in gene expression that are mediated by PKM2 in human cancers. Recent studies have demonstrated that post-translational modifications of PKM2, which include prolyl hydroxylation [27], lysine acetylation [38], cysteine oxidation [54], tyrosine phosphorylation [36], and sumoylation [63], play critical roles in PKM2-mediated

cancer metabolism and cell growth (Table 1). Identification of additional post-translational modifications of PKM2 may reveal yet other mechanisms by which PKM2 modulates cancer biology to promote disease progression. Because of the many important roles played by PKM2 as a pyruvate kinase, protein kinase, and transcriptional co-activator, the most effective therapeutic strategy for targeting the protein may be to prevent its synthesis, either at the transcriptional or translational level.

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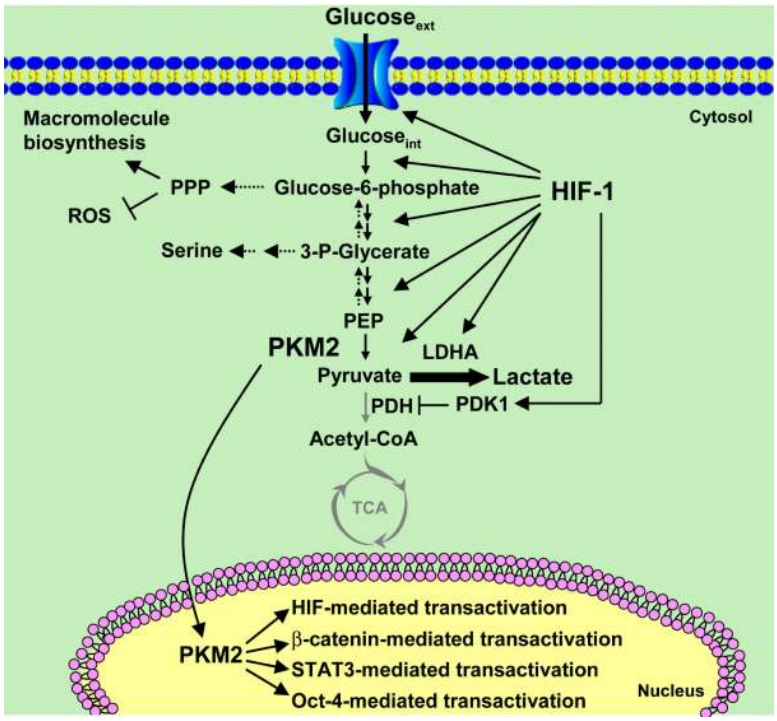

Figure 1. PKM2 promotes the Warburg effect and tumor growth

Expression of PKM2 promotes glucose uptake and lactate production and inhibits O_2 consumption in cancer cells, by coactivation of hypoxia-inducible factor 1 (HIF-1)-mediated gene transcription. Expression of the less active PKM2 dimer leads to the accumulation of upstream glycolytic metabolites, which include 3-phosphoglycerate (3-P-glycerate) that stimulates serine synthesis, and phosphoenopyruvate (PEP) and glucose-6-phosphate that stimulate the pentose phosphate pathway (PPP), thereby promoting macromolecule biosynthesis and inhibiting intracellular reactive oxygen species (ROS) generation. PKM2 also translocates into the nucleus to stimulate the activity of transcription factors including HIF-1, HIF-2, β-catenin, STAT3, and Oct-4, thereby increasing the expression of gene products that are required for tumor growth.

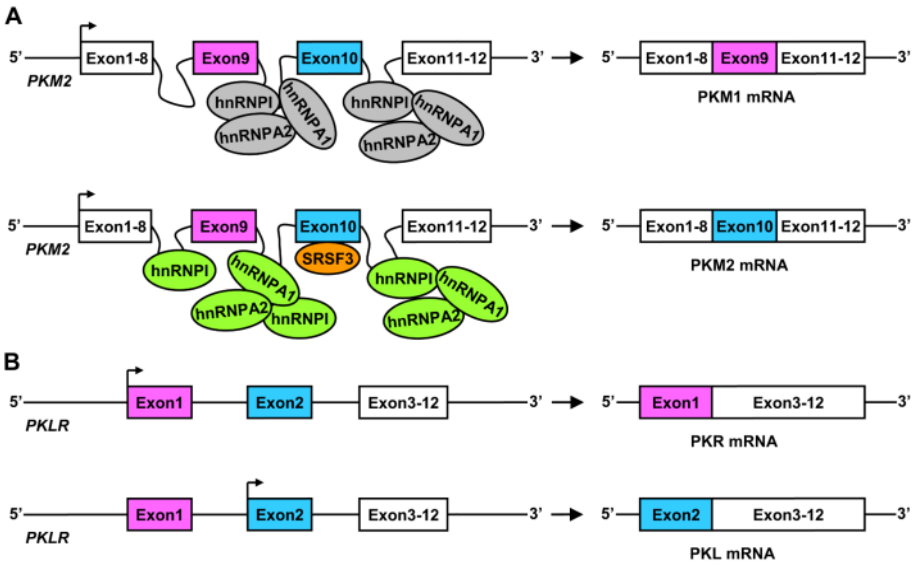

Figure 2. Regulation of expression of pyruvate kinase isoforms

(A) PKM1 and PKM2 are produced from *PKM2* gene through the alternative RNA splicing of the primary transcript. SRSF3 binds to an exonic splicing enhancer element in the exon 10 region to activate exon 10 inclusion, whereas high levels of heterogenous nuclear ribonucleoproteins (hnRNP) I, hnRNPA1, and hnRNPA2 (shown in green) bind to the intronic sequences flanking exon 9 and inhibit exon 9 inclusion in PKM2 mRNA. Low levels of hnRNPI, hnRNPA1, and hnRNPA2 (shown in grey) bind to intronic sequences flanking exon 10 and inhibit exon 10 inclusion in PKM1 mRNA. (B) PKR and PKL are encoded by *PKLR* gene and expressed under the control of tissue-specific promoters [64]. The transcription of PKR mRNA is initiated from exon 1 and exon 2 is spliced out of the mature mRNA, whereas the transcription of PKL mRNA is initiated from exon 2 of *PKLR*.

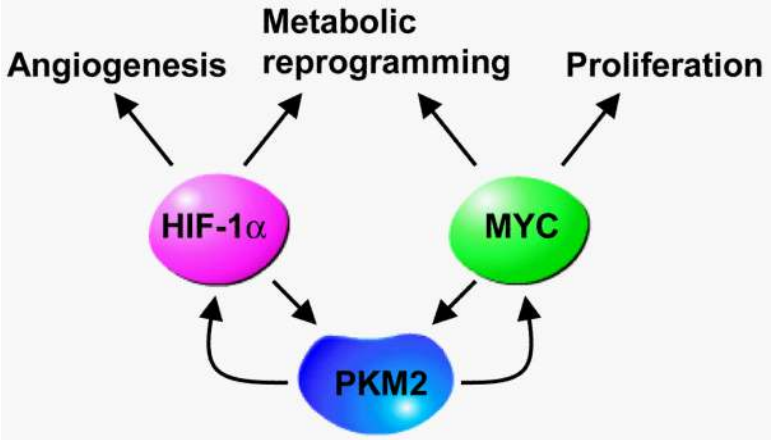

Figure 3. Feed-forward regulation of PKM2-HIF-1α and PKM2-MYC signaling

HIF-1 activates transcription of *PKM2*, leading to increased synthesis of PKM2 protein, which in turn promotes HIF-1-dependent gene transcription that mediates angiogenesis and metabolic reprogramming in cancer cells. Similarly, MYC controls *PKM2* gene transcription directly and PKM2 mRNA splicing indirectly. PKM2 also stimulates EGF-induced transcription of the β-catenin target gene *MYC*, which may regulate PKM2-mediated cell proliferation and metabolic reprogramming in cancer cells.

Table 1

Post-Translational Modifications of PKM2 and their Functions

Post-translational modification	Modified PKM2 residue(s)	Mechanism of modification	Functional consequence	Reference
Prolyl hydroxylation	Proline 403 and 408	PHD3	Increased interaction with HIF-1α	[27]
Phosphorylation	Tyrosine 105	FGFR1	Disruption of PKM2 tetramer formation	[36]
Acetylation	Lysine 333	PCAF	Interaction with Hsc70 and CMA	[38]
Oxidation	Cysteine 358	ROS	Disruption of PKM2 subunit association	[54]
Sumoylation	Not identified	PIAS3	Nuclear translocation	[63]

Table 2

PKM2-Coactivated Gene Transcription

Transcription Factor	PKM2-mediated gene transcription	Reference
HIF-1α	Induction of transcription of HIF-1 target genes *SLC2A1, LDHA, PDK1, HK1, VEGFA*	[27]
HIF-2α	Induction of transcription of HIF-2 target gene *VEGFA*	[27]
β-catenin	Induction of transcription of EGF-induced β-catenin target genes *CCND1, MYC*	[48]
STAT3	Induction of transcription of STAT3 target gene *MEK5*	[49]
Oct-4	Increase in Oct-4-mediated transactivation	[50]