PKM2

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The pyruvate kinase isoenzyme type M2 (PKM2) controls cell progression and survival through the regulation of key signaling pathways.

Keywords: pyruvate kinases ; cancer metabolism ; metabolic reprogramming ; long non-coding RNAs

1. PKM2 Transcription and Dynamic Regulation

Mammalian pyruvate kinase is expressed as one of four different isoforms (M1, M2, L, and R) encoded by two distinct genes (PKM and PKLR)[1]. These isoforms share similar features, where they catalyze the final step in glycolysis and exhibit the same primary structure containing four major domains: A, B, C, and N[2][3][4][5]. However, the PK isoforms differ in their enzymatic potential, allosteric regulation[6], amino acid sequence, tissue distribution[7][8], and contribution to health and disease[9][10]. PKM1 and PKM2 are both expressed from the *PKM* gene and conserved across vertebrates[11]. The amino acid sequence for PKM2 is highly similar between humans and mice at 82% similarity[12]. The *PKM* gene is located on chromosome 15 in humans and chromosome 9 in mice[13]. The human *PKM PKM* gene has 12 exons and 11 introns[14]. The two PK transcript isoforms M1 and M2 result from alternative splicing regulated by several spliceosomes including the heterogeneous nuclear ribonucleoprotein A1 and A2 (hnRNPA1 and hnRNPA2) and polypyrimidine tract binding protein (PTB)[15][16]. The inclusion of exon 9 and exclusion of exon 10 produces PKM1, whereas PKM2 includes exon 10 but not exon 9 [14]. Moreover, recent studies have shown that the insertion of exon 10 into the final PKM2 RNA is promoted through the action of the serine/arginine-rich splicing factor 3 (SRSF3)[17]. Both exon 9 and exon 10 are 167 base pairs and 56 amino acids in length[18],and the human PKM1 and PKM2 isoforms are both 531 amino acids long[3]. Consequently, the resulting M1 and the M2 isoforms differ by 22 amino acids located between amino acids 389 and 433 of the C-terminus domain[3]. The other two PK isozymes, PKL and PKR, are encoded by the PKLR gene, which is on chromosome 1 in humans and distinct from the *PKM* gene[19]. The human PKL and PKR isozymes still share approximately 71–72% amino acid similarity with PKM1 and PKM2, despite being transcribed from different genes[19]. Alternative splicing produces the R isoform, a 574 amino acid long protein that is strictly expressed in erythrocytes, and the L isoform, a 543 amino acid long protein that is highly expressed in the liver[1] and other tissues[20][21].

Even though all PK isoforms perform a similar enzymatic function, these isoforms differ in their kinetic properties and affinity towards phosphoenolpyruvate (PEP), while their affinity potential toward ADP remains comparable[4]. PKM2 exhibits lower enzymatic activity[22] and is the only isoform, to our knowledge, capable of existing in the enzymatically active “R-State” or inactive tetramer “T-State”, dimer, and monomer configurations[23]. This enables PKM2 to substantially alter its dynamics by existing in either the dimeric (high Km for PEP) and tetrameric forms (low Km for PEP)[24] to meet differential metabolic demands. The equilibrium of PKM2 configurations is tightly regulated by allosteric effectors, altering PKM2 kinetics and Km values for PEP[25]. In contrast, PKM1 predominantly exists in an active tetrameric form[26]. Similarly, the unphosphorylated PKL is considered active with higher affinity for PEP ($K_{0.5}$ = 0.3 mM) in comparison to the phosphorylated form ($K_{0.5}$ = 0.8 mM)[27]. However, under abnormal conditions, PKR was reported to exist in a mutated form with a tendency to dissociate into dimeric or monomeric configurations with altered Km value compared to unmutated enzyme[28]. Furthermore, PKM2 exhibits lower V_{max} compared to to PKM1[23], even though the fructose-1,6-bisphosphate (FBP) binding pockets of M1 and M2 are almost identical. The only reported difference is the presence of a glutamate residue in the M1 isoform instead of lysine in the M2 isoform[29]. Although minor, this difference was demonstrated to play a significant role in blocking the allosteric regulation of FBP in PKM1; however, it does not fully explain the kinetic variation between PKM isoforms.

Notably, the different PK isoforms are expressed in a tissue-specific manner that seems to be dependent upon energy requirements and the availability of nutrients[30]. For instance, PKL plays a role in gluconeogenic organs such as the kidney, liver, and small intestine[31], and can be phosphorylated and inhibited in response to high cellular levels of glucagon and ATP[30]. On the other hand, PKM1 is highly abundant amongst differentiated tissues (heart, brain, muscle, stomach, bone, skin, among others), where energy is produced and used rapidly[30]. PKM2, however, is expressed in the

embryonic stages initially and, in most cases, is gradually replaced by other PK isoforms[8]. Notably, it has been revealed that various differentiated tissues continue to express PKM2 across the lifespan[1][32]. PKM2 also differs from other PK isoforms through its ability to translocate to the nucleus and regulate the transcription of numerous genes with key functions in a plethora of cellular processes[33]. Additionally, while other PK isoforms exist in a stable tetrameric configuration, PKM2 may switch between the dimer or tetramer form in response to biological circumstances and metabolic needs[34]. This unique property of PKM2 allows for dynamic metabolic regulation, due in part to the variation in the affinity of the dimer and tetramer configurations of PKM2 to PEP.

2. Impact of PKM2 Mutations on Gene Expression

PKM2 expression, subcellular localization, and activity are regulated by several mechanisms. At the gene level, earlier studies have identified two missense mutations of PKM2 (H391Y and K422R) that could support the aggressive nature of cancer metabolism [35]. The two mutations are both specific to PKM2 but not PKM1 since they are encoded by exon 10 and were discovered in Bloom syndrome cells (H391Y) and a Bloom syndrome patient (K422R)[36]. Iqbal et al. transfected H1299 cells with either mutant or wild-type PKM2 mimicking the missense mutations, H391Y and K422R, and demonstrated that these missense mutations promote cancer proliferation through a variety of proposed metabolic alterations[35]. Cells transfected with the mutant PKM2 exhibited higher glucose uptake and lactate production, concomitant with a reduction in oxidative stress[35]. Moreover, in recent studies by Chen and colleagues, mutations in the exon 10 region of the PKM gene have been proposed to promote the translocation of PKM2 to the nucleus and have been associated with increased activity of the hypoxia-inducible factor 1-alpha (HIF-1α)[37]. HIF-1α is a well-established oxygen sensor in tumor cells and also a modulator of glycolysis and PKM2 expression through direct regulation of the c-Myc/hnRNP splicing axis to favor PKM2 expression[32]. In another study by M.V. Vander Heiden's group, the authors argued that since PKM2 is not required for the growth of several cancers, as demonstrated by earlier studies, loss-of-function mutations observed in some human tumors are not oncogenic but rather help to create a metabolic state that favors the proliferation of tumor cells[4]. Further efforts towards a comprehensive understanding of the metabolic and physiological consequences of PKM2 mutations as well as their associated clinical outcomes are needed.

As noted above, PKM2 is highly expressed during neonatal stages and phases of proliferation, a fact that may explain the increase in PKM2 expression in tumors given their highly proliferative nature and the associated metabolic requirements. For instance, the oncogenic transcription factor c-Myc enhances the expression of PKM2 through upregulating the expression of *PKM* spliceosomes [38]. Similarly, the activation of the rapamycin (RTK/PI3K/AKT/mTOR) signaling pathway in tumor suppressor (Tsc1/2) deficient mouse embryonic fibroblasts (MEF) leads to a cascade of events that upregulates the levels of HIF-1α and, subsequently, increased PKM2 levels. Comparable to c-Myc, mTOR activation can promote tumorigenesis and metabolic transformation[39] and was shown to be integral to oncogenesis, and the transition towards the Warburg effect[32].

A large number of factors have been shown to modulate the quaternary structure and physical configuration of PKM2, thus altering its enzymatic activity and subcellular localization[30][32]. For example, the cis-trans isomerization plays a critical role in mediating the non-enzymatic function of PKM2[40][41][42] through its conversion from a tetramer to a dimer or monomer. Although the tetrameric form is considered the active form and a higher tetramer/dimer ratio results in a higher conversion rate of PEP to pyruvate[1], PKM2 in tumor cells exists predominantly in the dimeric form and has been directly correlated with increased levels of lactate. It is likely that the high levels of dimer PKM2 relate to the "damming up effect" or the accumulation of glycolytic phospho-metabolites[1]. Meanwhile, the cis-trans isomerization of PKM2 and its transition between the tetramer and dimer forms can drastically alter its localization and functions. In tumors, the altered configuration of PKM2 provides cancer cells with the excess amino acids, nucleotides, and phospholipids needed for biosynthetic pathways during proliferation[1]. Notably, post-translational modifications play a key role in regulating the cis-trans isomerization of PKM2 and the associated metabolic consequences. For example, serine phosphorylation of PKM2 at position 37 (Ser-37) by ERK1/2 facilitates the recruitment of peptidyl-prolyl cis-trans isomerase NIMA-interacting 1 (PIN1), which mediates PKM2 cis-trans isomerization[40][42]. This conformational change exposes the nuclear localization signal (NLS) and results in the translocation of PKM2 to the nucleus, a process that requires the binding of PKM2 to importin α5 [40].

In addition to the cis-trans regulation of PKM2, several other factors were demonstrated to alter the quaternary structure and physical configuration of PKM2[1][3], PKM2 subcellular localization, and, subsequently, its functions. For instance, it is well established that phenylalanine acts as an allosteric inhibitor for both PKM1[43] and PKM2[23], thus reducing their affinity to PEP. FBP, on the other hand, is an allosteric regulator that promotes PKM2 tetramerization[44] resulting in PKM2 activation and the subsequent increase in glucose utilization[45]. Unlike PKM2, PKM1 lacks the regulatory effect of FBP due to differences in the orientation of the FBP-activating loop[3], which results in a significant reduction in PKM1's ability

to sense glucose. Accordingly, PKM2 missense mutations could potentially alter glucose uptake in cancer cells[35]. Moreover, SAICAR (succinyl-5′-phosphoribosyl-5-amino-4-imidazole carboxamide) and serine have also been identified as independent stimulants of PKM2 activity[46][47]. SAICAR allosterically stimulates PKM2 in a nutrient-dependent manner[47], while serine acts as an allosteric activator and ligand of PKM2 and both may play a critical role in the metabolic transformation required in oncogenesis[46]. These allosteric regulators could aid cancer cells in the metabolic transformation, allowing them to thrive in an environment limited in nutrients[22].

Moreover, post-translational modifications of PKM2 through oxidation, phosphorylation, and acetylation can also modify its activity, conformation, and localization[22]. Phosphorylation of PKM2 at tyrosine 105 residue (Tyr-105) stabilizes the dimer configuration, leading to inactivation of PKM2's glycolytic activity[48]. A similar reduction in glycolytic function was also seen in response to PKM2 oxidation at cysteine (Cys)-358, which results in the entrance of glucose into the pentose phosphate pathway[49]. PKM2 is sensitive to oxidation by several oxidants including nitric oxide (NO), endothelial NO synthase (eNOS), and hydrogen peroxide (H_2O_2), all of which were demonstrated to be capable of regulating PKM2's activity and its subcellular localization[8]. Notably, the redox regulation of PKM2 was shown to have substantial effects on both cancerous and non-cancerous metabolic outcomes. Therefore, it is imperative to consider redox homeostasis when investigating PKM2, although more research is still needed for a better understanding of the clinical impact of the full scope of oxidants and their regulation of PKM2 in metabolic transformation. It is worth noting, however, that alterations in PKM2 activity through oxidation in tumors facilitate cancer cells' adaptation to oxidative stress through multiple distinct pathways. Post-translational modifications that reduce PKM2 activity, such as the oxidation of Cys-358[49] and the dessuccinylation of Lys-498[50] residues, increase the accumulation of glycolytic metabolites that promote glucose entrance into the pentose phosphate pathway, which generates reduced equivalents in the form of NADPH to clear excessive oxidant accumulation and maintain cancer cell survival. In addition, recent studies have shown that the PKM2-specific Cys-424 plays a crucial role in its conformational change and the transition between the tetrameric and dimeric forms. Mutation of this residue to leucine resulted in a higher tetramer to dimer ratio and resistance to oxidative stress-induced oxidation and inhibition of PKM2[51].

3. Regulation of PKM2 Subcellular Distribution

The functions of PKM2 and its location within the cells are heavily dependent on its final assembled structure[1]. In the cytosol, PKM2 exhibits both tetrameric and dimeric isoforms and mainly converts PEP to pyruvate and controls a key regulatory step in glycolysis[52]. However, within the nucleus, PKM2 exists in the dimeric form and is involved in the regulation of gene expression [33]. The nuclear translocation of PKM2 is shown to be dependent upon a variety of complex protein–protein interactions. Recently, it has been demonstrated that the phosphorylation of PKM2 at Ser-37 by extracellular signal-regulated kinase 2 (ERK2) could ultimately allow the proper conformational change required for PKM2 translocation into the nucleus[40], a process that requires the binding of PKM2 to importin α5[40]. The nuclear accumulation of PKM2 promotes the phosphorylation of histone H3, which can promote mitotic chromatin condensation[53], and upregulates the transcription of cell-cycle-regulating genes including MYC and CCND1[40]. Additionally, nuclear PKM2 was shown to play a key role in breast cancer cell proliferation and angiogenesis through modulation of epidermal growth factor receptor (EGFR) signaling and its downstream miR-148a and miR-152 genes. Furthermore, evidence suggests a direct interaction between PKM2 and the p65 subunit of nuclear factor kappa light chain enhancer of activated B cells (NF-κB), a well-established factor involved in cancer development and progression[54]. Furthermore, the nuclear translocation of the dimeric form of PKM2 was shown to be responsible for mediating HIF-1α function in the transition towards aerobic glycolysis[55]. According to recent studies, the interaction between PKM2 and HIF-1α leading to the activation of the latter's transcriptional activity is dependent upon PKM2 hydroxylation at proline residues 403 and 408 by prolyl hydroxylase 3 (PHD3)[56]. Importantly, this interaction between the two proteins underscores the role of PKM2 in several aspects of cancer biology, given the role of HIF-1α in tumor progression, angiogenesis, invasion, metastasis, as well as adaptation to oxidative stress caused by exposure to chemicals and radiation[57][58][59].

In the nucleus, PKM2 was also shown to play a critical role in regulating β-catenin expression and downstream signaling with profound effects on the cell cycle, survival, and proliferation of tumor cells. Increased β-catenin levels have been implemented as a potential contributor to cancer development and proliferation[33][60]. The precise mechanisms by which PKM2 interacts and regulates β-catenin have been described previously[33][61][62] and were suggested to be essential to cancer cell proliferation[33][62]. Yang et al. identified that EGFR-activated ERK phosphorylates PKM2 but not PKM1, promoting PKM2 binding to importin α5 and its subsequent nuclear translocation[40]. Within the nucleus, PKM2-mediated phosphorylation of β-catenin at Y333 results in the subsequent induction of *c-Myc*[40]. Supportively, in another study, the activation of EGFR signaling resulted in PKM2-dependent β-catenin phosphorylation at Y333 and subsequent upregulation of c-Myc expression[63]. Consistent with these findings, in a more recent study; PKM2 silencing reduced the

nuclear accumulation of β-catenin[64]. Likewise, the downregulation of PKM2 expression in Hep3B cells suppressed β-catenin activity and promoted its proteolytic degradation[65]. Conversely, the overexpression of PKM2 negatively modulated β-catenin signaling through a mechanism that was proposed to be dependent on the upregulation of miR-200a[61]. Interestingly, in thyroid cancer (TC) cells, the interaction between PKM2 and β-catenin was recently demonstrated to be dependent upon AMPK activation [66]. In this study, the binding of AMPK to PKM2 promoted β-catenin nuclear translocation and was deemed necessary for the migration of TC cells. Notably, findings from this study suggest that PKM2/β-catenin interaction and perhaps phosphorylation occur in the nucleus as PKM2 deficiency suppressed the nuclear accumulation of β-catenin, but not AMPK. Regardless, when combined, these studies emphasize the importance of the regulatory actions that PKM2 can exert on the β-catenin pathway. Moreover, the evidence shows that the induced nuclear activity and translocation of PKM2 can result in diverse cellular and metabolic outcomes, warranting continued exploration beyond its known cytosolic functions.

Outside the nucleus, PKM2 has been detected within other subcellular fractions including the mitochondria[67][68] and exosomes [69][70]. Under increasing oxidative stress, PKM2 can translocate to the mitochondria, where it can inhibit apoptosis through the phosphorylation and stabilization of BCL2[67]. Likewise, glucose deprivation can lead to PKM2 succinylation and its mitochondrial translocation in HCT116 cells. Subsequently, this translocation resulted in an increase in ATP generation and mitochondrial permeability through inhibiting voltage-dependent anion channel 3 (VDAC3) ubiquitination, promoting cancer cell survival[68]. Recent studies have also identified a novel mechanism through which PKM2 regulates cancer cells' interaction with their microenvironment through exosome release. Indeed, Wei and colleagues demonstrated that PKM2 could enable the release of exosomes through the phosphorylation of synaptosome-associated protein 23 (SNAP-23) and subsequent formation of the SNARE complex[69]. Exosomes have been shown to play critical roles in tumorigenesis through their role in promoting growth and expansion[71]. Taken together, these findings emphasize the crucial role that PKM2 may exhibit as a key regulator of various aspects of tumorigenesis through its ability to modulate multiple signaling pathways at different subcellular locations.

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