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Cellular Control Mechanisms that Regulate Pyruvate Kinase M2 Activity and Promote Cancer Growth

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Nearly a century ago, Otto Warburg made the seminal observation that tumors metabolize glucose more rapidly than normal tissue and do so even in the presence of oxygen [1,2]. Since this observation, numerous studies have addressed how cancer cells enhance glucose uptake and the mechanisms by which this additional glucose is utilized [3,4]. Several lines of evidence suggest that cancer cells reprogram their metabolic networks through coordinated regulation of enzyme expression and activity to enhance anabolic processes. One process that influences expression of metabolic enzymes important in cancer is alternative splicing. Among the alternatively spliced genes expressed in cancer cells is the embryonic isoform of pyruvate kinase, PKM2 [5]. This observation was pioneered by Erich Eigenbrodt and is now considered as a major node in the regulation of cancer metabolism [6,7]. In the following commentary, we describe the mechanisms by which the activity of PKM2 is controlled in tumors to facilitate cell growth, work that has stemmed from Eigenbrodt's efforts exploring the oncogenic role of this glycolytic isozyme.

Pyruvate kinase (PK) is a glycolytic enzyme that converts phosphoenolpyruvate (PEP) and ADP into pyruvate and ATP. This reaction is both a rate-limiting step and the final step in glycolysis [6]. In mammals, there are two genes that encode four PK isozymes [8]. Alternative promoter utilization of the *Pklr* gene allows for PKL expression in the liver and PKR in red blood cells. Like the tissue-specific enzymes PKL and PKR, the PKM1 isozyme is expressed in differentiated, somatic tissues (e.g. neurons, muscle). Alternative splicing of the *Pkm* gene can also result in expression of the embryonic PKM2 isoform characteristic of proliferating cells, the immune system, and notably, cancer cells [9].

PKM1 exists in a highly active homotetrameric form, whereas PKM2 can exist in either an active homotetrameric form or a less active dimeric or monomeric form [10]. A significant body of evidence now suggests that PKM2 expression is required to rewire cellular metabolism from that of a quiescent, homeostatic state to that of a proliferative state [3,6,11-14]. Indeed, PKM1 and the tissue-specific PK isoforms are universally replaced by PKM2 in cancer cells. Mechanistically, this occurs because the activity of PKM2 can be regulated in response to various metabolic inputs. When the activity of PKM2 is low, the terminal step in glycolysis is proposed to become a bottleneck that results in elevated concentrations of upstream glycolytic metabolites. Consequently, this

increased metabolite abundance is predicted to change the chemical potential of anabolic reactions that branch off of glycolysis and thereby promotes the diversion of glycolytic flux into such pathways (Figure 1) [3,15]. Notably, these pathways produce many of the biosynthetic building blocks required for making cells, including the pentose phosphate pathway (PPP; which produces ribose and NADPH for nucleotide and lipid biosynthesis) and the serine synthesis pathway (which produces the amino acids serine and glycine, and ultimately contributes to nucleic acid biosynthesis via the one carbon pool).

The PKM isozymes are each composed of 531 amino acids and only differ in 21 amino acids concentrated within the region spanning residues 381 to 435 of the protein. This region is encoded by either exon 9 (PKM1) or exon 10 (PKM2), whose inclusion into the final mRNA transcript is mutually exclusive [5]. While most of the sequence is conserved between the two isozymes (including the active site, which is identical), many of the residues involved in allosteric control are different. Indeed, various metabolic inputs can regulate these allosteric sites thereby governing catalytic and regulatory activity. This is in contrast to the residues involved in post-translational control, which are conserved between the isozymes. Notably, however, these two processes are highly intertwined since post-translational control of PKM2 is only likely to occur upon tetramer dissociation as the modifiable residues are buried in the tetrameric structure. In this review, we will discuss the residues whose allosteric or post-translational modification control the activity of PKM2, the mechanisms by which such control is mediated and the functional output on cellular survival and proliferation.

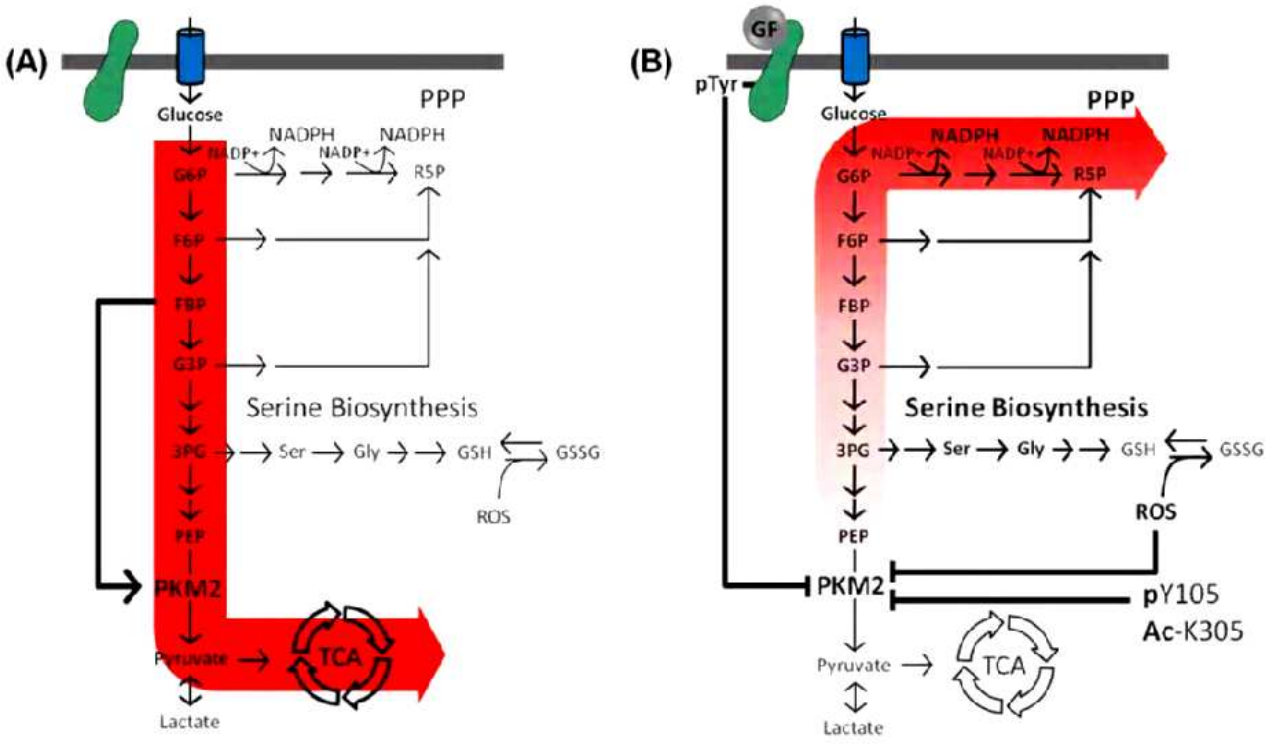

Figure 1. PKM2 Activity Influences the Direction of Glycolytic Flux. *(A) When PKM2 is activated by FBP, glycolytic intermediates are preferentially diverted into the TCA cycle. (B) Inhibition of PKM2 (by tyrosine-phosphorylated growth factor receptors (pTyr), ROS or by direct phosphorylation or acetylation-mediated degradation) promotes the redirecting of glycolytic intermediates into biosynthetic pathways.*

Allosteric Control of PKM2

In order to appreciate why cancer cells select for PKM2, it is critical to understand what makes PKM2 unique from the other tissue-specific PK enzymes. Unlike PKM1, PKM2 is allosterically activated by the glycolytic intermediate fructose-1,6-bisphosphate (FBP) [16]. Interaction with FBP facilitates the transition of PKM2 into the highly active tetrameric state and drives the enzymatic conversion of PEP into pyruvate, serving as a classical feed-forward regulatory loop. In this way, cells that ex-

press PKM2 can activate the enzyme when glycolytic intermediates (and specifically FBP) accumulate to a degree that anabolic pathways are unable to handle the overload (Figure 1).

If PKM2 can be turned on, then it is reasonable to assume that it can also be turned off in order to promote anabolic shunting of glycolytic intermediates and thus growth. Indeed, using a proteomic screen for phospho-tyrosine (pTyr)-binding proteins, it was found that PKM2 binds directly to (pTyr)-peptides, where binding results in the release of FBP and inhibition of PKM2 enzymatic activity.[12] The interaction between pTyr-peptides and PKM2 is mediated by lysine 433 (K433), a residue unique to PKM2; position 433 in PKM1 is glutamate. Moreover, K433E mutation renders PKM2 insensitive to allosteric control by FBP or pTyr-peptides. Based on these findings, we demonstrated that PKM2, and not PKM1, has the ability to undergo dynamic regulation by FBP when a tyrosine kinase signal transduction pathway is activated.

Furthermore, by switching PK expression from the M2 to the M1 isoform, our lab also demonstrated that PKM2 facilitates aerobic glycolysis [11]. Cancer cell lines engineered to express PKM1 produce less lactate, consume more oxygen and are less tumorigenic in nude mouse xenografts. These results suggest that the metabolic phenotype conferred by PKM2 provides a selective growth advantage for tumor cells *in vivo*. We suspect that this mechanism evolved to ensure that proliferating tissues in a developing organism only use glucose for growth when they are appropriately activated by growth factor receptor protein-tyrosine kinases.

In addition to FBP, numerous other metabolites have also been reported to allosterically modulate the activity of PKM2. Some of these include: alanine, several amino acids with hydrophobic side chains (*i.e.* methionine, phenylalanine, valine, leucine, isoleucine), serine, cysteine, saturated and mono-unsaturated fatty acids and 3,3´,5-triiodo-L-thyronine (T3) [7,17-19]. Alanine is a non-essential amino acid and can be synthesized from pyruvate or branched-chain amino acids (*e.g.* valine, leucine, isoleucine). Alanine modulates PKM2 activity by lowering its affinity for PEP and by increasing the concentration of FBP necessary for tetramerization [7]. Collectively, this provides a negative feedback loop where high concentrations of alanine (or branched chain amino acids) are able to inhibit pyruvate formation, and subsequently, alanine biosynthesis. Lastly, T3 has also been reported as an allosteric mediator of PKM2 and specifically as an inhibitor of the monomer-to-tetramer transition. T3 can bind to the monomeric form of PKM2 and thereby prevent its association into the tetrameric form [17]. These results are particularly intriguing in light of the role that T3 plays in coordinating growth and development, metabolism, body temperature, and heart rate [20]. The extent to which amino acids and T3 influence PK activity *in vivo* however remains to be determined.

Post-Translational Control of PKM2

In addition to numerous allosteric mechanisms that modulate PKM2 activity, studies from several laboratories have recently revealed post-translational modes of PKM2 regulation. Based on observations that PK orthologues in many organisms (from bacteria to humans) are modified by oxidants [21-24], the effect of oxidative stress on PKM2 activity and function was recently examined.[14] Pathways frequently mutated in cancer, such as Ras and PI3K, are thought to result in increased accumulation of reactive oxygen species (ROS), and the control of intracellular ROS concentrations is critical for cell proliferation and survival.[25] Notably, exposure of cancer cell lines to various oxidizing agents/environments resulted in a decrease in PKM2 activity [14]. Reversal of oxidation, with a strong reducing agent, restored PKM2 activity, indicating that the oxidative effects on PKM2 activity are reversible.

Through a series of biochemical assays, it was then established that an oxidative environment caused PKM2 inhibition through the oxidation of one residue in particular, Cys358. Importantly, Cys358 oxidation impairs association of the active tetrameric form of PKM2 (and thus PKM2 activity), and mutation of Cys358 to serine (C358S) could reverse the effects of oxidative stress on PKM2 inhibition. Mechanistically, the inhibition of PKM2 by ROS creates a bottleneck in glycolytic flux that leads to the diversion of glycolytic flux into the oxidative arm of the PPP and, thereby, generates reducing potential (in the form of NADPH and ultimately reduced glutathione) for the detoxification of ROS (Figure 1). Moreover, cancer cell lines in which endogenous PKM2 was

replaced with the C358S mutant exhibited increased sensitivity to oxidative stress and impaired tumor formation *in vivo*.

An interesting question that arises from this observation is why have cells selected for the terminal enzyme in glycolysis as the redox checkpoint, as opposed to upstream enzymes. One potential explanation comes from another recent observation which illustrated that some types of cancer cells select for amplified glycolytic flux through the serine biosynthetic pathway [26-28]. In doing so, glycolytic metabolites can then be used to synthesize the amino acids glycine (by way of serine) and cysteine, which are building blocks of glutathione. As such, inhibiting a glycolytic enzyme upstream of the breakpoint enzyme in serine biosynthesis (phosphoglycerate dehydrogenase) may also impair glutathione biosynthesis. Collectively, these data indicate that PKM2 confers advantages to cancer cells by reprogramming metabolic functions associated with promoting anabolic processes (oxidative PPP and serine biosynthesis), which allows them to sustain anti-oxidant responses and thereby support cell survival under oxidative stress.

In addition to the inhibition of PKM2 activity by cysteine oxidation, several studies have revealed other modes of post-translationally-mediated PKM2 inactivation: namely, phosphorylation and acetylation-mediated degradation [29,30]. In an effort to understand if tyrosine kinase signaling regulates PKM2 activity, Hitosugi *et al.* found that oncogenic forms of fibroblast growth factor receptor type 1 (FGFR1) phosphorylate PKM2 at tyrosine 105 (Y105) [29]. Indeed, FGFR1-mediated Y105 phosphorylation (pY105) inhibits PKM2 activity by disrupting the binding of FBP. The authors proposed that this is achieved in an inter-subunit manner, where pY105 of a PKM2 molecule will cause the release of FBP via interaction with K433 in a neighboring PKM2 subunit within the tetramer [12] Furthermore, they demonstrated that pY105 is commonly observed in cancer cell lines, where mutation of Y105 to phenylalanine (Y105F) leads to decreased cell proliferation under hypoxic conditions, increased oxidative phosphorylation with reduced lactate production, and reduced tumor growth in xenografts in nude mice. Collectively, these findings suggest that pY105 regulates PKM2 to provide a metabolic, growth-promoting advantage to tumor cells. Lv, *et al.*, demonstrated another mechanism to reduce the activity of PKM2; that is, acetylation-mediated and lysosome-dependent degradation [30]. In particular, they show that PKM2 is acetylated on lysine 305 (K305) and that this acetylation is stimulated by high glucose concentration. Ectopic expression of an acetylation mimetic, in which K305 is mutated to glutamine (K305Q), results in the accumulation of glycolytic intermediates and promotes cell proliferation and tumor growth. These results reveal an acetylation-induced degradation-dependent mode of regulation of PKM2.

The recent resurgence of interest in cancer metabolism can be attributed in part to the observation that many of the signaling pathways affected by genetic mutations control nutrient uptake and utilization [31]. Erich Eigenbrodt's pioneering studies on PKM2 paved the way for much of the current work on an enzyme that plays a critical role in the reprogramming of cellular metabolism and the promotion of tumor growth. Methods to harness this understanding for the treatment of disease are well underway [14,32,33] and may ultimately help to translate Warburg's original observations into methods for cancer diagnosis and therapy.

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