ABBS

Review

Acetylation control of metabolic enzymes in cancer: an updated version

Wei Huang[1,2,3]†, Zhen Wang[1,2,4]†, and Qun-Ying Lei[1,2]*

[1]Key Laboratory of Molecular Medicine, Ministry of Education, Department of Biochemistry and Molecular Biology, Shanghai Medical College, Fudan University, Shanghai 200032, China
[2]Molecular and Cell Biology Lab, Institutes of Biomedical Sciences, Fudan University, Shanghai 200032, China
[3]Department of Biological Sciences, Harper Cancer Research Institute, University of Notre Dame, South Bend, IN 46617, USA
[4]School of Life Science, Fudan University, Shanghai 200032, China
†These authors contributed equally to this work.
*Correspondence address. Tel: +86-21-54237935; Fax: +86-21-54237853; E-mail: qlei@fudan.edu.cn

Metabolic reprogramming is one of the critical features in cancer. Tumor cells preferentially utilize glycolysis instead of oxidative phosphorylation in the presence of oxygen, namely 'Warburg Effect'. Recent studies have provided new insights into the Warburg effect, elucidating metabolic-dependent and independent mechanisms of metabolic enzymes regulated by post-translational modifications and providing further evidence for the critical role of these tricks in cancer metabolism and tumorigenesis. Of particular interest, we summarized the latest advances in both the metabolic and the non-metabolic functions of metabolic enzymes via the acetylation regulation in the Warburg effect. In addition, their potential roles in cancer metabolism therapy will also be briefly discussed.

Introduction

Cancer is a leading cause of death worldwide. Different underlying mechanisms have been found in multiple cancers. However, only metabolic reprogramming was frequently referred to represent the common feature in almost all kinds of carcinomas. Initially noted by Otto Warburg [1,2], metabolic reprogramming in cancers was called Warburg effect in the later years. Compared with normal cells, cancer cells preferentially utilize glycolysis instead of oxidative phosphorylation even in the presence of normal oxygen pressure (**Fig. 1**). Meeting the requirement for rapid proliferation of cancer cells, metabolic reprogramming could provide more building blocks, such as nucleotides and amino acids, through glycolysis and give the cancer cells an appropriate microenvironment by producing more lactate. More and more studies supported the notion that metabolic

reprogramming is a hallmark of cancer, and drugs targeting metabolic pathways probably become the most potential therapeutic approach in multiple cancers.

A number of studies have proved that cancer cells implement the metabolic reprogramming by regulating transcription or post-translational modification (PTM) of metabolic enzymes [4]. Of all the regulating mechanisms, protein acetylation could target and regulate almost all the metabolic enzymes, and this kind of regulation is conserved from bacteria to human beings [5–7]. Lysine acetylation is a reversible dynamic process that is catalyzed by competing enzymes, i.e. protein lysine acetyltransferases (KATs, commonly termed as histone acetyltransferases) and deacetylases (commonly known as histone deacetylases). Recent studies have demonstrated that acetylation may serve as a wide bridge between the extracellular nutrient status and intracellular metabolic pathways under physiological conditions, or even more important in pathological states. Several reviews have discussed the acetylation regulation of metabolic enzymes in cancer [6–9]. Lately, studies on metabolite kinase functions as protein kinase made protein moonlighting re-emerge as a hot topic, called non-metabolic functions *in vivo* [10–12]. In this review, we will briefly summarize the regulation of both metabolic and non-metabolic functions of metabolic enzymes by acetylation in cancer in the past 3 years, and also discuss their potential roles in cancer therapy.

Acetylation Control of Metabolic Enzymes in Metabolic-Dependent Manner

As mentioned before, the Warburg effect is an essential hallmark of cancer. According to the Warburg effect, glucose uptake is significantly increased in tumor cells compared with that of normal cells, but the ATP production is not in line with the oxidative phosphorylation of this high glucose does [2,13,14]. In another words, some of the absorbed
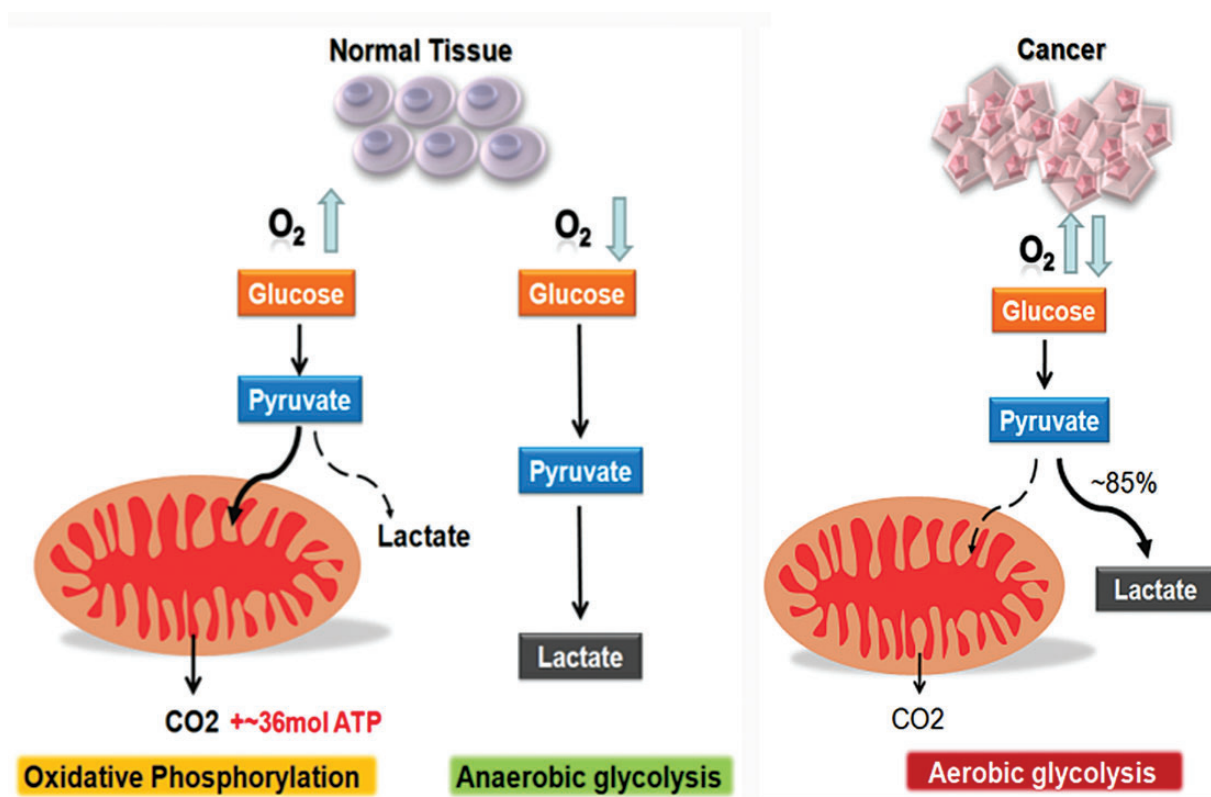
Figure 1. Different metabolic features in normal and cancerous tissues [3] Under normal oxygen pressure, glucose preferentially goes through mitochondrial oxidative phosphorylation in normal tissues, which leads to the production of CO_2 and ATP. Under low oxygen pressure, cells switch away from oxidative phosphorylation to glycolysis to generate more lactate (anaerobic glycolysis) and less ATP. For cancer tissues, cells preferentially metabolize most glucose to lactate even under normal oxygen conditions (aerobic glycolysis).

glucose is not used to produce ATP in cancer cells. Acting as the main source of nutrition in resting cells, glucose is used for ATP production through the tricarboxylic acid cycle (TCA cycle). But in dividing cells, glucose is also the source of carbon, free energy, and metabolite to synthesize fatty acid and other biomass to support cell proliferation. Therefore, a reasonable explanation for the Warburg effect is that tumor cells need to convert some glucose to macromolecular precursors, such as acetyl-CoA and ribose for fatty acid and nucleotide synthesis, respectively. Here comes the question, how could oxidative phosphorylation and glycolysis be coordinately regulated to promote cell growth in cancer cells, which is also the key step in metabolic reprogramming in cancer [15,16]. Many published reviews have discussed the acetylation control of metabolic enzymes and metabolic reprogramming in cancer [6,8,9]. Here, we will focus on the acetylation regulation of several important enzymes in cancer.

Acetylation of PKM2[K305] accumulates the metabolic intermediates for tumor growth

Pyruvate kinase (PK), which catalyzes the last step of glycolysis, is a key enzyme for glucose fermentation. PK catalyzes the transfer of a phosphate group from phosphoenolpyruvate

(PEP) to ADP, generating one molecule of pyruvate and one molecule of ATP. There are four isoforms of PK in mammals: L, R, M1 and M2 encoded by two genes, *PKL/R* (pyruvate kinase, liver and red blood cells) and *PKM* (pyruvate kinase, muscle). The L and R isoforms, which are mainly expressed in liver or red blood cells, are encoded by different transcript variants of *PKL/R* [17,18]. PKM1 and PKM2 are encoded by different transcript variants of *PKM*. PKM1 is expressed in organs that are strongly dependent upon a high rate of energy regeneration, such as muscle and brain. PKM2 is expressed in some differentiated tissues, such as lung, fat tissue, retina, pancreatic islets, as well as in all cells with a high rate of nucleic acid synthesis, such as normal proliferating cells, embryonic cells, and especially tumor cells [19–24]. In the beginning, it has been concluded that the expression of PKM1 switches to PKM2 during cancer development based on the comparison of PKM1/PKM2 expression ratio between cancer cell lines and human muscle [25], and this process was tightly regulated by MYC oncoprotein [26]. However, other evidence showed that there is only an up-regulation of PKM2, but not a shift in PKM1 to PKM2 during tumorigenesis [27]. Bluemlein *et al.* performed a pure quantification of PKM1/2 splice isoforms in samples of human cancers, tissues, and certain cell lines by

using mass spectrometry. They found that PKM2 was the predominant isoform in all analyzed samples, which was not due to PK splicing. Consistently, Yang *et al.* reported that EGFR (epidermal growth factor receptor) activation up-regulates PKM2 expression, but not PKM1 expression, by coordinated regulating nuclear factor-kappa B (NF-kB)-enhanced PKM gene transcriptional expression and PTB-dependent splicing of pre-mRNA. Their findings highlighted the significance of the cooperation between the EGFR and NF-kB pathways in PKM2 up-regulation and metabolic reprogramming [28].

As pyruvate, the product of PK, serves as the substrate of pyruvate dehydrogenase in TCA cycle, it is reasonable to speculate that inhibition of PK may repress pyruvate metabolism through mitochondria along with enhanced aerobic glycolysis. Christofk *et al.'s* [29] work supported this hypothesis. In 2008, they found that inhibition of PKM2 enzymatic activity by binding directly to tyrosine-phosphorylated peptides led to the shift of metabolism from energy production to anabolic processes, which affected cellular metabolism. An important point should be noted here is that cancer cells are not in a shortage of energy when they turned into 'Warburg effect' metabolism model because of the existence of alternative pathways to supply the substrate for TCA cycle and oxidative phosphorylation, such as those from glutamine metabolism.

Recently, Lv *et al.* [30] also reported that acetylation of PKM2 may be involved in this metabolism switch. In this report, it was found that p300/ (CREB binding protein) associated factor (PCAF) acetylates PKM2 at lysine 305, which decreases its enzymatic activity. Moreover, acetylation of $PKM2^{K305}$ enhances its interaction with HSC70 and promotes its lysosome-dependent degradation via CMA (chaperone-mediated autophagy) under high glucose stimulation. The decrease of PKM2 enzymatic activity and its protein level results in the accumulation of glycolytic intermediate metabolites upstream of PKM2, including FBP (fructose-1, 6-bisphosphate) and G6P (glucose-6-phosphate). In line with the metabolic alteration, ectopically expressed acetylation mimetic $PKM2^{K305Q}$ promotes cell proliferation and tumor growth. This result supports a very good example that metabolic reprogramming, i.e. switch from ATP production to building block preparation, could be tightly regulated by acetylation at metabolic enzymes. Considering the tumorigenesis effect of acetylation at K305 of PKM2, drugs repressing PKM2 acetylation by targeting the acetyltransferase or deacetylases have potential therapeutic value for some cancers.

Decreased levels of $LDHA^{K5}$ acetylation implicate in human pancreatic cancer initiation

LDH (lactate dehydrogenase) converts pyruvate, the final product of glycolysis, to lactate when oxygen is absent or there is shortage, and it also performs the reverse reaction during the Cori cycle in liver, converting lactate to glucose.

LDHA belongs to the lactate dehydrogenase family and prefers to catalyze pyruvate to lactate. When converting pyruvate to lactate, LDH also generates one NAD^+ which is necessary for the glycolysis reaction catalyzed by glycerol-3-phosphate dehydrogenase (GAPDH). It has been reported that the protein level of LDHA is frequently elevated in multiple tumors [31–34], and the potential explanation is that MYC and HIF, two transcription factors, could target *LDHA* gene directly [35,36]. Inhibition of LDHA by either RNA interference or pharmacological agents blocks tumor progression *in vivo* [37]. Recently, Zhao *et al.* [38] found that acetylation regulates both enzymatic activity and degradation of LDHA. Interestingly, the opposite results were observed although LDHA and PKM2 were regulated by acetylation in a similar way. In normal cells, acetylation of $LDHA^{K5}$ inhibits its enzymatic activity and promotes its degradation via CMA, keeping the metabolism in normal cells away from 'tumor style'. In human pancreatic cancers, decreased levels of $LDHA^{K5}$ acetylation result in activation of LDHA enzymatic activity and inhibition of LDHA degradation, eventually promotes cancer cell growth and migration. Besides, lactate, the product of LDHA, can affect the microenvironment, and subsequently promotes the communication between cancer cells and stromal cells.

In addition, we found that $LDHA^{K5}$ acetylation is decreased in pancreatic cancer, but not liver cancer, indicating that acetylation at K5 of LDHA might be a marker for some types of cancers, such as pancreatic cancer (**Fig. 2**). Given that elevated LDHA is detected in various cancers, it has been used to monitor treatment of some cancers because of its correlation with poor prognosis and chemotherapy/radiotherapy resistance. The specific antibody for $LDHA^{K5}$ acetylation may serve as a valuable tool for the diagnosis of some cancers. We further speculate that $LDHA^{K5}$ acetylation coupled with positron emission tomography/computed tomography would be a potential early diagnostic marker for pancreatic cancer. Regarding the inhibitory effect of K5 acetylation on LDHA, drugs promoting LDHA acetylation by targeting the acetyl transferase or deacetylase should inhibit LDHA, therefore may have potential therapeutic value for cancers with high LDHA activity. Collectively, our recent study not only suggested a novel mechanism of LDHA regulation, but also provided a potential early diagnostic maker and therapeutic target for pancreatic cancer.

Acetylation competes with ubiquitylation to stabilize ACLY for tumor growth in lung cancer

As long-chain fatty acids are building blocks of bilayer cell membranes, phospholipids, and precursors of lipid second messengers, *de novo* lipid synthesis plays an important role in dividing cells. Actually, lipogenesis is also a part of metabolic reprogramming in cancer. In the reaction of fatty acid elongation, Acetyl-CoA serves as the only substrate. ACLY
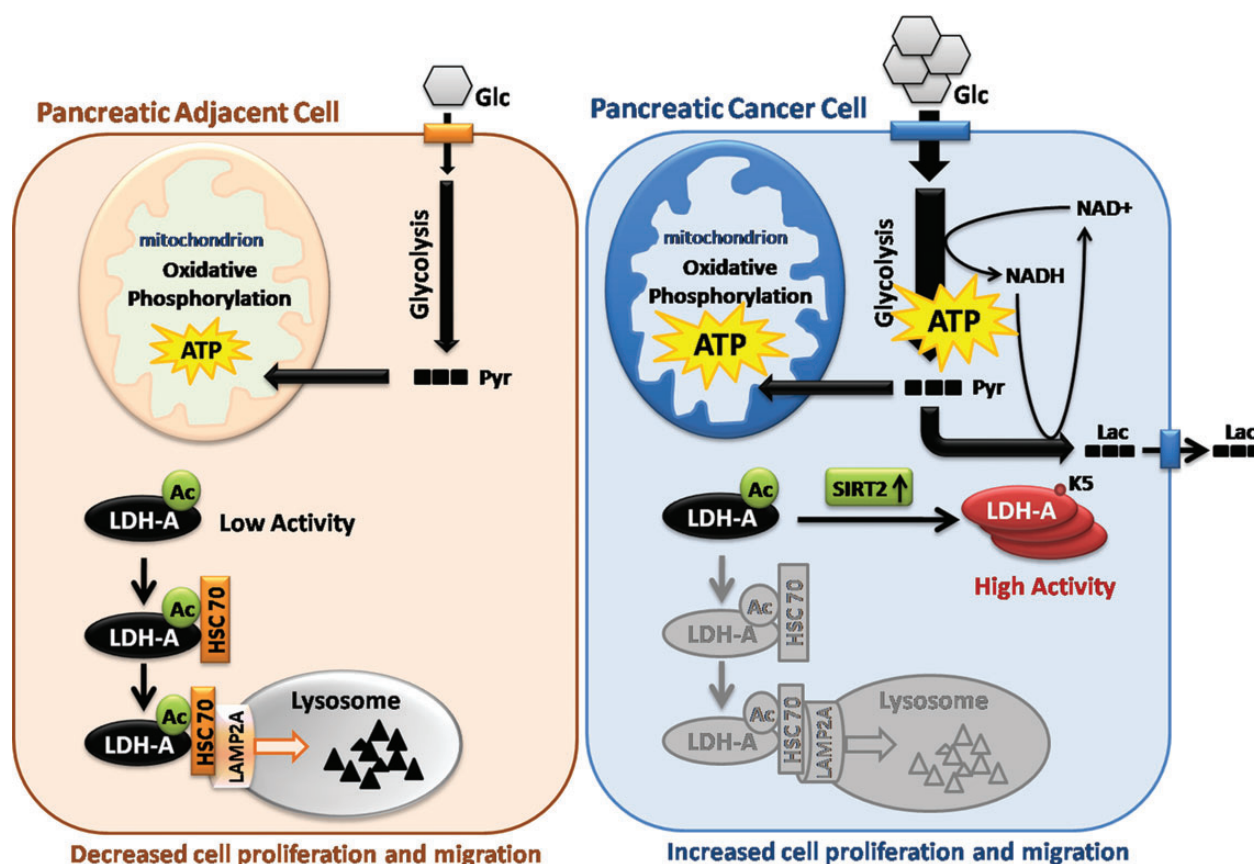
Figure 2. Acetylation of LDHA at K5 inhibits its enzymatic activity and promotes its lysosomal degradation via CMA [38] In adjacent normal tissues, LDHA acetylation inhibits its activity and promotes its binding with HSC70. In pancreatic cancer tissues, LDHA could be deacetylated by Sirt2 which is elevated in cancer tissues. Deacetylation of LDHA leads to the elevated enzymatic activity and accumulated protein level, thereby accelerating glycolysis and lactate production, leading to increased cell proliferation and migration. Glc, glucose; Pyr, pyruvate; Lac, lactate; Ac, acetylation.

(ATP-citrate lyase), which is the crucial enzyme for the synthesis of cytosolic acetyl-CoA, provides the majority of cytosolic Ac-CoA in most tissues. Therefore, ACLY becomes the key enzyme to integrate glucose and lipid metabolism. The regulation of ACLY mainly happens at transcription level by sterol regulatory element-binding protein 1 [39]. Several studies have demonstrated that the enzymatic activity of ACLY is regulated by the phosphatidylinositol-3-kinase/ Akt pathway [40−43]. Besides the phosphorylation on ACLY, Lin *et al.* reported that ACLY is acetylated at lysines 540, 546, and 554 in response to high glucose. Acetylation at these three lysines leads to the inhibition of ubiquitylation-dependent degradation, resulting in enhancing lipogenesis, increasing cell proliferation, and promoting *in vivo* tumor growth (**Fig. 3**) [44]. Notably, acetylation of ACLY^{3K} is also significantly elevated in lung carcinomas, suggesting that acetylation at ACLY^{3K} may take part in lung cancer initiation or development. Considering ACLY is up-regulated or activated in multiple types of cancer, inhibition of ACLY by chemical inhibitors or RNAi may suppress tumor cell proliferation and induce differentiation *in vitro* and *in vivo* [40,45]. Our study not only uncovered the novel mechanism

on the regulation of ACLY, but also provided the possibility that drugs inhibiting the acetylated ACLY may merit exploration as a therapeutic agent for cancer.

Mn-SOD and IDH2 acetylation regulates tumorigenesis through reactive oxygen species

As discussed above, the deregulation of acetylation in some metabolic enzymes results in tumorigenesis directly or indirectly. Reactive oxygen species (ROS) are chemically reactive molecules containing oxygen, which are constantly generated and eliminated in the biological system [46]. Much evidence suggested that cancer cells exhibit increased intrinsic ROS stress, which can lead these cancer cells to fatal lesions by damaging cellular proteins, lipids, and DNA [47−49]. Mutations in *superoxide dismutase 2* (*SOD2*) gene are associated with aging, cancer, and other human diseases [50]. Based on these research, SOD2 may play a critical role in ROS balance. Lately, three groups reported that SOD2 is deacetylated by Sirt3 to activate its enzymatic activity and prevent cells from oxidative damage by ROS [51−53]. Isocitrate dehydrogenase 2 (IDH2) has recently been shown to have an antioxidant function. Sirt3 deacetylates and
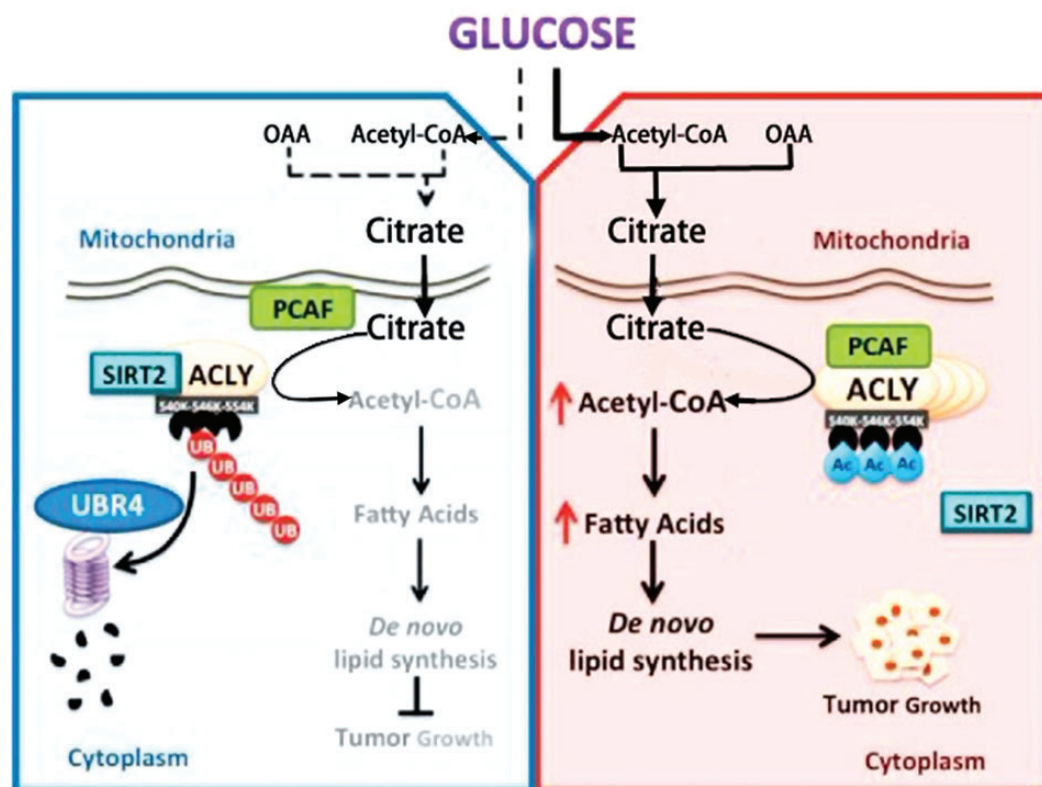
Figure 3. Acetylation of ACLY^{3K} increases lipid synthesis to promote tumor growth and is elevated in human lung cancer [44] Acetylation at 3K protects ACLY from ubiquitin-mediated proteasome degradation in response to high glucose. This elevated protein level of ACLY contributes to *de novo* lipogenesis and promotes tumor cell proliferation.

activates IDH2, increasing NADPH levels in mitochondria and protects the cells from oxidative stress [54]. These all give cancer cells further advantage to resist oxidative stress.

Acetylation Control of Metabolic Enzymes in Non-metabolic-Dependent Manner

There are reviews which summarized metabolic enzymes with other functions relating to cancer indirectly [55,56]. Recently, the study on metabolite kinase functions as a protein kinase made protein moonlighting re-emerge as a hot topic, called non-metabolic functions *in vivo*. In the following sections, we will discuss the non-metabolic functions and the potential therapeutic roles of metabolic enzymes regulated by acetylation in cancer.

Mitogenic and oncogenic signals promote acetylation of PKM2^{K433} to function as a protein kinase

PKM2 is the most famous metabolic enzyme that has non-metabolic functions in metabolic reprogramming, which has already been discussed in several articles [10,12,57,58]. PKM2 converts PEP to pyruvate by transferring a phosphate from PEP to ADP when it acts as a metabolic enzyme. However, recently, Yang *et al.* [11] demonstrated that

PKM2 functions as a protein kinase to modify histone phosphorylation in its non-metabolic manner, which is critical for it to regulate gene expression and tumorigenesis epigenetically. They reported that PKM2 binds to histone H3 directly to phosphorylate histone H3 at T11 under EGFR activation. Furthermore, H3^{T11} phosphorylation and nuclear PKM2 expression are positively correlated. Moreover, T11 phosphorylation also correlates well with the glioma malignancy grades and prognosis. Gao *et al.* [10] reported that PKM2 acts as a protein kinase in nucleus to phosphorylate STAT3 at Y705 which could finally activate the transcription of MEK5. The transfer of a phosphate in this reaction is very similar to protein phosphorylation catalyzed by protein kinases. When PKM2 acts as a protein kinase in nucleus, PKM2 transfers a phosphate from PEP, instead of ADP, to STAT3. As PKM2 can switch between the dimer and the tetramer, their experiments suggested that the dimeric PKM2 is an active protein kinase [10]. On account of the role that PKM2 plays in promoting cell proliferation, this study revealed an important link between metabolism alteration and gene expression in cancer. The transfer of a phosphate or other molecule from an intermediate to another happens in many metabolic processes. As a result, this study gave us a revelation that metabolic enzymes could also induce this

kind of transfer between intermediates and proteins to carry out the reaction which is similar to PTM.

Besides protein kinase activity, PKM2 also plays other moonlighting roles in nucleus. In 2011, Luo *et al.* [57] reported that PKM2 functions as a co-activator of hypoxia-inducible factor 1 (HIF1). In that study, they found that hydroxylation on proline[403/408] of PKM2 by PHD3 enhances the binding between PKM2 and HIF1 [57]. As the transcription of *PKM2* is activated by HIF-1, PKM2 may participate in a positive feedback loop by binding with HIF1 and promoting its transactivation. At the end of the same year, Yang *et al.* [12] reported another non-metabolic function of PKM2 in nucleus. They demonstrated that activation of EGFR induces translocation of PKM2, but not PKM1 into the nucleus. Locating in the nucleus, PKM2 binds to c-Src-phosphorylated Y333 of β-catenin to activate its transcription activity. They also revealed the positive correlations between c-Src activity, β-catenin phosphorylation, and PKM2 nuclear accumulation in human glioblastoma specimens. In 2012, the same group established the mechanism of PKM2 nuclear translocation and reported that EGFR-activated ERK2 phosphorylates PKM2 at Ser 37 [58]. Consequently, phosphorylated PKM2 recruits PIN1 for *cis–trans* isomerization of PKM2, which promotes PKM2 binding to importin alpha5 and translocating to the nucleus. Nuclear translocation is required by all non-metabolic functions of PKM2, especially protein kinases. Recently, Lv *et al.* [59] found that PKM2 is acetylated by p300 acetylase at K433 in response to mitogenic and oncogenic signals. More importantly, compared with PKM1, only PKM2 could be acetylated at K433. K433 acetylation in PKM2 promotes its dimer formation by interfering its binding with fructose-1, 6-bisphosphate (FBP), the allosteric activator of PKM2, and leads to its nuclear accumulation which is well correlated with its protein kinase activity to promote STAT3[Y705] phosphorylation. Consistently, acetylation-mimetic PKM2[K433Q] mutant promotes tumor cell growth *in vivo* and *in vitro* (**Fig. 4**). PKM2 acetylation at K433 is enriched in human breast cancer was also observed. Yang *et al.* reported that EGFR activation could phosphorylate PKM2 at Ser 37, which promotes PKM2 translocating to the nucleus by binding to importin alpha5. Their findings demonstrated the importance of PKM2 nuclear functions in Warburg effect and tumorigenesis [58]. Considering the functions of PKM2[S37] phosphorylation and PKM2[K433] acetylation in PKM2 nuclear translocation, a crosstalk might exist between these two modification pathways. As Zhang *et al.* [60] reported a functional crosstalk between glycogen phosphorylase acetylation and phosphorylation, it is very likely that PKM2[K433] acetylation could affect its phosphorylation in regulating its nuclear translocation. It will be interesting to determine whether these two regulations operate separately or sequentially to affect PKM2 functions.

Multiple non-metabolic dependent functions of GAPDH is strictly regulated by acetylation

GAPDH is an important metabolic enzyme that catalyzes the sixth step of glycolysis that aims to decompose glucose. The non-metabolic functions of nuclear GAPDH were found much earlier than PKM2. In 1993, Zeev Ronai reviewed that at least four modified glycolytic enzymes can localize in the nucleus to bind DNA. They are LDH, phosphoglycerate kinase, aldolase, and GAPDH, respectively [61]. Locating in the nucleus, GAPDH can bind nucleic acids to mediate RNA nuclear export [62], protect mRNA from degradation [63], and repair DNA lesions [64]. Several studies have shown that GAPDH can bind telomeres directly and protect telomeres from chemotherapy-induced rapid degradation [65,66]. Elevated expression and nuclear accumulation of GAPDH were found to be the early critical events during apoptosis [67]. Recently, some studies have demonstrated that GAPDH is implicated in immunity and senescence [68–70]. Modification of GAPDH by S-nitrosylation, acetylation, and phosphorylation supports the potential mechanisms on nuclear translocation of GAPDH [68–70]. During apoptosis, the activated inducible nitric oxide synthase or neuronal NOS could generate nitric oxide S-nitrosylating GAPDH and promote its binding with Siah1, an E3-ubiquitin-ligase with a nuclear localization signal [68]. Nuclear GAPDH is acetylated at lysine 160 by the acetyltransferase p300/CBP, which in turn stimulates the acetylation and catalytic activity of p300/CBP. Under this feedback loop, targets of p300/CBP, such as p53, are activated and subsequently regulate cell death [71]. Besides, acetylated GAPDH by the overexpression of acetyltransferase PCAF leads to its translocation to the nucleus to promote apoptosis [70]. It is also reported that Sirt1 restrains GAPDH entering nuclear via direct interaction with GAPDH in a deacetylase activity independent manner [72]. Hence, Sirt1 depletion triggers nuclear translocation of cytosolic GAPDH even without apoptotic stress [72]. Besides, GAPDH functions as a co-activator for androgen receptor (AR) which plays an important role in the development and progression of prostate cancer. GAPDH enhances AR transactivation, independent of its glycolytic activity in prostate cancer cells. Therefore, instead of in the nucleus, the formation of a GAPDH·AR complex in the cytoplasm is essential for GAPDH to enhance AR transactivation [73,74].

Perspective

Genetically, the more functions a protein has, the more complex regulations it needs to work properly. PTM is the perfect way to perform this function. By covalent addition of functional groups to proteins, proteolytic cleavage of
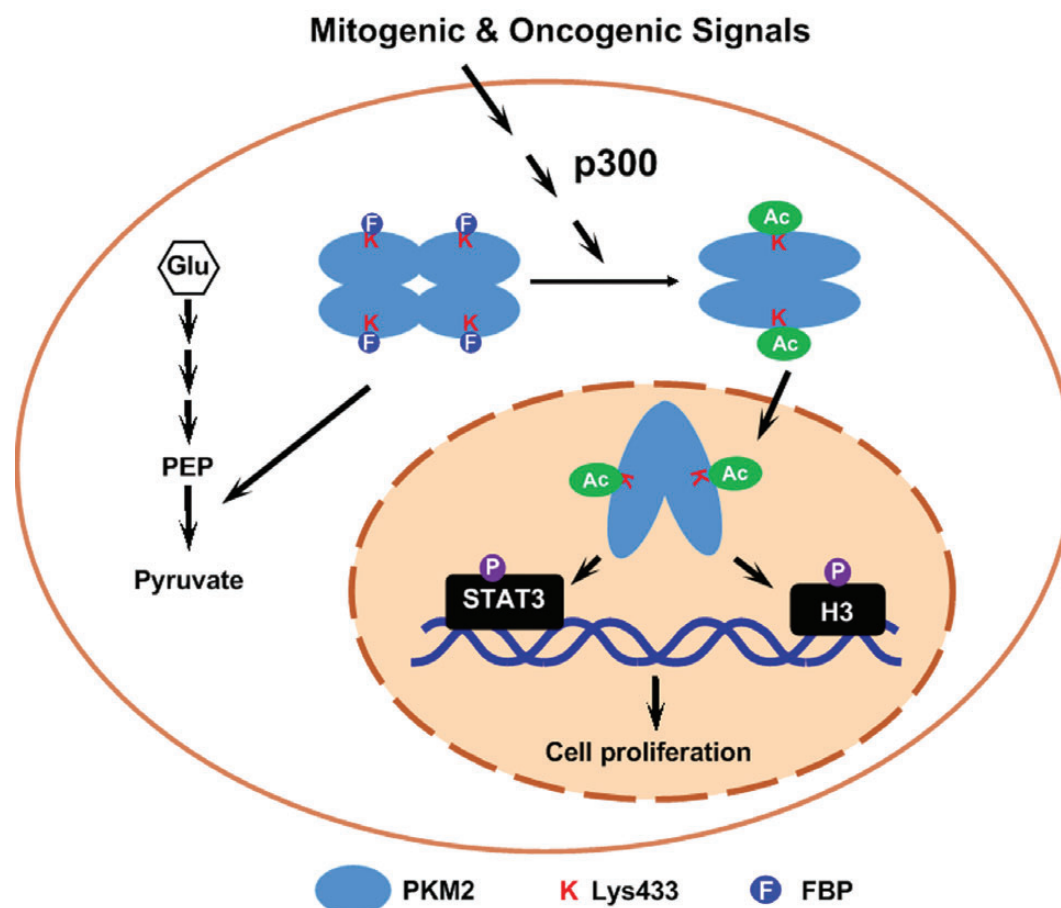
Figure 4. Acetylation regulation of PKM2 in cancer cells [59] PKM2 is acetylated by p300 acetyltransferase at K33, which is unique in PKM2 for its binding with fructose-1, 6-bisphosphate directly. Acetylation prevents PKM2 activation by interfering its binding with FBP and promotes the nuclear accumulation and protein kinase activity of PKM2. Acetylation-mimetic PKM2^{K433Q} mutant promotes cell proliferation and tumorigenesis.

regulatory subunits or degradation of entire proteins, PTM makes one protein work as various proteins in different PTM status. It is much easier and quicker than synthesis of new proteins. The metabolic and non-metabolic functions of metabolic enzymes discussed above are usually involved with PTM, especially when translocation is needed. PTM controls and regulates the multiple functions of metabolic enzymes precisely. Recently, great progress has been made on lysine acetylation regulation of non-nuclear proteins. In addition, studies have shown that acetylation regulation of metabolic enzymes plays an essential role in cancer. While the picture of acetylation in cancer is now emerging, more questions are raised. First, besides acetylation, other PTM such as phosphorylation, ubiquitylation also play important roles in metabolic enzymes' regulation. It is very interesting to explore how this new emerging rival (acetylation) cross-talk with phosphorylation and/or ubiquitylation in cancer metabolism. Secondly, the key step now is to expand these studies further in animal models to define the important role of acetylation of these metabolic enzymes in cancer development. Such studies will not only provide the fine-tune acetylation control of cancer metabolism *in vivo* but also the

animal models for small-molecular drug research. Thirdly, due to the acetylation levels of specific sites are responses to diverse signal; it is more rational to develop cancer therapy drugs targeting on specific acetylation sites of important metabolic enzymes in cancer. For example, PKM2^{K305} is stimulated at the presence of high glucose and involves with its PK activity; while PKM2^{K433} is increased in response to mitogenic and oncogenic signals and positively correlated with its protein kinase activity. Regarding cancer type variety, distinct strategies targeting PKM2 PK or/and protein kinases should be take into account, respectively. In addition, it was reported that the senescence effect of GAPDH is regulated by its substrate NAD^+ and G3P [67]. If the substrate of metabolic enzymes always involves in regulation of moonlighting, it would be much easier to design inhibitor or activator for the non-metabolic function than to target the lysine acetylases and deacetylases which have multiple substrates. This makes moonlighting of metabolic enzymes a better target in therapy. Addressing these questions will not only provide the insight into the underlying mechanisms of cancer cell metabolism but also help to develop potential new targets for cancer therapy.

Funding

References

1. Warburg O. On respiratory impairment in cancer cells. Science 1956, 124: 269–270.

2. Warburg O. On the origin of cancer cells. Science 1956, 123: 309–314.

3. Lin R, Zhou X, Huang W, Zhao D, Lv L, Xiong Y and Guan KL, et al. Acetylation control of cancer cell metabolism. Curr Pharm Des 2013, PMID: 23859620.

4. Vander Heiden MG, Cantley LC and Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science 2009, 324: 1029–1033.

5. Wang Q, Zhang Y, Yang C, Xiong H, Lin Y, Yao J and Li H, et al. Acetylation of metabolic enzymes coordinates carbon source utilization and metabolic flux. Science 2010, 327: 1004–1007.

6. Xiong Y and Guan KL. Mechanistic insights into the regulation of metabolic enzymes by acetylation. J Cell Biol 2012, 198: 155–164.

7. Zhao S, Xu W, Jiang W, Yu W, Lin Y, Zhang T and Yao J, et al. Regulation of cellular metabolism by protein lysine acetylation. Science 2010, 327: 1000–1004.

8. Guan KL and Xiong Y. Regulation of intermediary metabolism by protein acetylation. Trends Biochem Sci 2011, 36: 108–116.

9. Xu W, Li Y, Liu C and Zhao S. Protein lysine acetylation guards metabolic homeostasis to fight against cancer. Oncogene 2013, 163: 1–7.

10. Gao X, Wang H, Yang JJ, Liu X and Liu ZR. Pyruvate kinase M2 regulates gene transcription by acting as a protein kinase. Mol Cell 2012, 45: 598–609.

11. Yang W, Xia Y, Hawke D, Li X, Liang J, Xing D and Aldape K, et al. PKM2 phosphorylates histone H3 and promotes gene transcription and tumorigenesis. Cell 2012, 150: 685–696.

12. Yang W, Xia Y, Ji H, Zheng Y, Liang J, Huang W and Gao X, et al. Nuclear PKM2 regulates beta-catenin transactivation upon EGFR activation. Nature 2011, 480: 118–122.

13. Gatenby RA and Gillies RJ. Why do cancers have high aerobic glycolysis? Nat Rev Cancer 2004, 4: 891–899.

14. Kim JW and Dang CV. Cancer's molecular sweet tooth and the Warburg effect. Cancer Res 2006, 66: 8927–8930.

15. Hanahan D and Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011, 144: 646–674.

16. McKeehan WL. Glycolysis, glutaminolysis and cell proliferation. Cell Biol Int Rep 1982, 6: 635–650.

17. Kanno H, Fujii H, Hirono A and Miwa S. cDNA cloning of human R-type pyruvate kinase and identification of a single amino acid substitution (Thr384––Met) affecting enzymatic stability in a pyruvate kinase variant (PK Tokyo) associated with hereditary hemolytic anemia. Proc Natl Acad Sci USA 1991, 88: 8218–8221.

18. Tani K, Fujii H, Tsutsumi H, Sukegawa J, Toyoshima K, Yoshida MC and Noguchi T, et al. Human liver type pyruvate kinase: cDNA cloning and chromosomal assignment. Biochem Biophys Res Commun 1987, 143: 431–438.

19. Brinck U, Eigenbrodt E, Oehmke M, Mazurek S and Fischer G. L- and M2-pyruvate kinase expression in renal cell carcinomas and their metastases. Virchows Arch 1994, 424: 177–185.

20. Corcoran E, Phelan JJ and Fottrell PF. Purification and properties of pyruvate kinase from human lung. Biochim Biophys Acta 1976, 446: 96–104.

21. Eigenbrodt E, Reinacher M, Scheefers-Borchel U, Scheefers H and Friis R. Double role for pyruvate kinase type M2 in the expansion of phosphometabolite pools found in tumor cells. Crit Rev Oncog 1992, 3: 91–115.

22. Mazurek S, Boschek CB, Hugo F and Eigenbrodt E. Pyruvate kinase type M2 and its role in tumor growth and spreading. Semin Cancer Biol 2005, 15: 300–308.

23. Reinacher M and Eigenbrodt E. Immunohistological demonstration of the same type of pyruvate kinase isoenzyme (M2-Pk) in tumors of chicken and rat. Virchows Arch B Cell Pathol Incl Mol Pathol 1981, 37: 79–88.

24. Tolle SW, Dyson RD, Newburgh RW and Cardenas JM. Pyruvate kinase isozymes in neurons, glia, neuroblastoma, and glioblastoma. J Neurochem 1976, 27: 1355–1360.

25. Christofk HR, Vander Heiden MG, Harris MH, Ramanathan A, Gerszten RE, Wei R and Fleming MD, et al. The M2 splice isoform of pyruvate kinase is important for cancer metabolism and tumour growth. Nature 2008, 452: 230–233.

26. David CJ, Chen M, Assanah M, Canoll P and Manley JL. HnRNP proteins controlled by c-Myc deregulate pyruvate kinase mRNA splicing in cancer. Nature 2009, 463: 364–368.

27. Bluemlein K, Gruning NM, Feichtinger RG, Lehrach H, Kofler B and Ralser M. No evidence for a shift in pyruvate kinase PKM1 to PKM2 expression during tumorigenesis. Oncotarget 2011, 2: 393–400.

28. Yang W, Xia Y, Cao Y, Zheng Y, Bu W, Zhang L and You MJ, et al. EGFR-induced and PKCε monoubiquitylation-dependent NF-κB activation upregulates PKM2 expression and promotes tumorigenesis. Mol Cell 2012, 48: 771–784.

29. Christofk HR, Vander Heiden MG, Wu N, Asara JM and Cantley LC. Pyruvate kinase M2 is a phosphotyrosine-binding protein. Nature 2008, 452: 181–186.

30. Lv L, Li D, Zhao D, Lin R, Chu Y, Zhang H and Zha Z, et al. Acetylation targets the M2 isoform of pyruvate kinase for degradation through chaperone-mediated autophagy and promotes tumor growth. Mol Cell 2011, 42: 719–730.

31. Goldman RD, Kaplan NO and Hall TC. Lactic dehydrogenase in human neoplastic tissues. Cancer Res 1964, 24: 389–399.

32. Koukourakis MI, Giatromanolaki A, Sivridis E, Bougioukas G, Didilis V, Gatter KC and Harris AL. Lactate dehydrogenase-5 (LDH-5) overexpression in non-small-cell lung cancer tissues is linked to tumour hypoxia, angiogenic factor production and poor prognosis. Br J Cancer 2003, 89: 877–885.

33. Koukourakis MI, Giatromanolaki A, Sivridis E, Gatter KC and Harris AL. Lactate dehydrogenase 5 expression in operable colorectal cancer: strong association with survival and activated vascular endothelial growth factor pathway—a report of the Tumour Angiogenesis Research Group. J Clin Oncol 2006, 24: 4301–4308.

34. Koukourakis MI, Kontomanolis E, Giatromanolaki A, Sivridis E and Liberis V. Serum and tissue LDH levels in patients with breast/gynaecological cancer and benign diseases. Gynecol Obstet Invest 2009, 67: 162–168.

35. Kim JW, Zeller KI, Wang Y, Jegga AG, Aronow BJ, O'Donnell KA and Dang CV. Evaluation of myc E-box phylogenetic footprints in glycolytic genes by chromatin immunoprecipitation assays. Mol Cell Biol 2004, 24: 5923–5936.

36. Semenza GL, Roth PH, Fang HM and Wang GL. Transcriptional regulation of genes encoding glycolytic enzymes by hypoxia-inducible factor 1. J Biol Chem 1994, 269: 23757–23763.

37. Le A, Cooper CR, Gouw AM, Dinavahi R, Maitra A, Deck LM and Royer RE, et al. Inhibition of lactate dehydrogenase A induces oxidative stress and inhibits tumor progression. Proc Natl Acad Sci USA 2010, 107: 2037–2042.

38. Zhao D, Zou SW, Liu Y, Zhou X, Mo Y, Wang P and Xu YH, et al. Lysine-5 acetylation negatively regulates lactate dehydrogenase A and is decreased in pancreatic cancer. Cancer Cell 2013, 23: 464–476.

39. Kim HS, Patel K, Muldoon-Jacobs K, Bisht KS, Aykin-Burns N, Pennington JD and van der Meer R, et al. SIRT3 is a mitochondria-localized tumor suppressor required for maintenance of mitochondrial integrity and metabolism during stress. Cancer Cell 2010, 17: 41–52.

40. Bauer DE, Hatzivassiliou G, Zhao F, Andreadis C and Thompson CB. ATP citrate lyase is an important component of cell growth and transformation. Oncogene 2005, 24: 6314–6322.

41. Berwick DC, Hers I, Heesom KJ, Moule SK and Tavare JM. The identification of ATP-citrate lyase as a protein kinase B (Akt) substrate in primary adipocytes. J Biol Chem 2002, 277: 33895–33900.

42. Migita T, Narita T, Nomura K, Miyagi E, Inazuka F, Matsuura M and Ushijima M, et al. ATP citrate lyase: activation and therapeutic implications in non-small cell lung cancer. Cancer Res 2008, 68: 8547–8554.

43. Potapova IA, El-Maghrabi MR, Doronin SV and Benjamin WB. Phosphorylation of recombinant human ATP:citrate lyase by cAMP-dependent protein kinase abolishes homotropic allosteric regulation of the enzyme by citrate and increases the enzyme activity. Allosteric activation of ATP:citrate lyase by phosphorylated sugars. Biochemistry 2000, 39: 1169–1179.

44. Lin R, Tao R, Gao X, Li T, Zhou X, Guan KL and Xiong Y, et al. Acetylation stabilizes ATP-citrate lyase to promote lipid biosynthesis and tumor growth. Mol Cell 2013, 51: 506–518.

45. Hatzivassiliou G, Zhao F, Bauer DE, Andreadis C, Shaw AN, Dhanak D and Hingorani SR, et al. ATP citrate lyase inhibition can suppress tumor cell growth. Cancer Cell 2005, 8: 311–321.

46. Dickinson BC and Chang CJ. Chemistry and biology of reactive oxygen species in signaling or stress responses. Nat Chem Biol 2011, 7: 504–511.

47. Gupta SC, Hevia D, Patchva S, Park B, Koh W and Aggarwal BB. Upsides and downsides of reactive oxygen species for cancer: the roles of reactive oxygen species in tumorigenesis, prevention, and therapy. Antioxid Redox Signal 2012, 16: 1295–1322.

48. Irani K, Xia Y, Zweier JL, Sollott SJ, Der CJ, Fearon ER and Sundaresan M, et al. Mitogenic signaling mediated by oxidants in Ras-transformed fibroblasts. Science 1997, 275: 1649–1652.

49. Waris G and Ahsan H. Reactive oxygen species: role in the development of cancer and various chronic conditions. J Carcinog 2006, 5: 14.

50. Miao L and St. Clair DK. Regulation of superoxide dismutase genes: implications in disease. Free Radic Biol Med 2009, 47: 344–356.

51. Chen Y, Zhang J, Lin Y, Lei Q, Guan KL, Zhao S and Xiong Y. Tumour suppressor SIRT3 deacetylates and activates manganese superoxide dismutase to scavenge ROS. EMBO Rep 2011, 12: 534–541.

52. Qiu X, Brown K, Hirschey MD, Verdin E and Chen D. Calorie restriction reduces oxidative stress by SIRT3-mediated SOD2 activation. Cell Metab 2010, 12: 662–667.

53. Tao R, Coleman MC, Pennington JD, Ozden O, Park SH, Jiang H and Kim HS, et al. Sirt3-mediated deacetylation of evolutionarily conserved lysine 122 regulates MnSOD activity in response to stress. Mol Cell 2010, 40: 893–904.

54. Someya S, Yu W, Hallows WC, Xu J, Vann JM, Leeuwenburgh C and Tanokura M, et al. Sirt3 mediates reduction of oxidative damage and prevention of age-related hearing loss under caloric restriction. Cell 2010, 143: 802–812.

55. Bhardwaj A and Wilkinson MF. A metabolic enzyme doing double duty as a transcription factor. Bioessays 2005, 27: 467–471.

56. Jeffery CJ. Moonlighting proteins: old proteins learning new tricks. Trends Genet 2003, 19: 415–417.

57. Luo W, Hu H, Chang R, Zhong J, Knabel M, O'Meally R and Cole RN, et al. Pyruvate kinase M2 is a PHD3-stimulated coactivator for hypoxia-inducible factor 1. Cell 2011, 145: 732–744.

58. Yang W, Zheng Y, Xia Y, Ji H, Chen X, Guo F and Lyssiotis CA, et al. ERK1/2-dependent phosphorylation and nuclear translocation of PKM2 promotes the Warburg effect. Nat Cell Biol 2012, 14: 1295–1304.

59. Lv L, Xu YP, Zhao D, Li FL, Wang W, Sasaki N and Jiang Y, et al. Mitogenic and oncogenic stimulation of K433 acetylation promotes PKM2 protein kinase activity and nuclear localization. Mol Cell 2013, 52: 1–13.

60. Zhang T, Wang S, Lin Y, Xu W, Ye D, Xiong Y and Zhao S, et al. Acetylation negatively regulates glycogen phosphorylase by recruiting protein phosphatase 1. Cell Metab 2012, 15: 75–87.

61. Ronai Z. Glycolytic enzymes as DNA binding proteins. Int J Biochem 1993, 25: 1073–1076.

62. Sirover MA. New insights into an old protein: the functional diversity of mammalian glyceraldehyde-3-phosphate dehydrogenase. Biochim Biophys Acta 1999, 1432: 159–184.

63. Bonafe N, Gilmore-Hebert M, Folk NL, Azodi M, Zhou Y and Chambers SK. Glyceraldehyde-3-phosphate dehydrogenase binds to the AU-Rich 3′ untranslated region of colony-stimulating factor-1 (CSF-1) messenger RNA in human ovarian cancer cells: possible role in CSF-1 posttranscriptional regulation and tumor phenotype. Cancer Res 2005, 65: 3762–3771.

64. Krynetski EY, Krynetskaia NF, Bianchi ME and Evans WE. A nuclear protein complex containing high mobility group proteins B1 and B2, heat shock cognate protein 70, ERp60, and glyceraldehyde-3-phosphate dehydrogenase is involved in the cytotoxic response to DNA modified by incorporation of anticancer nucleoside analogues. Cancer Res 2003, 63: 100–106.

65. Demarse NA, Ponnusamy S, Spicer EK, Apohan E, Baatz JE, Ogretmen B and Davies C. Direct binding of glyceraldehyde 3-phosphate dehydrogenase to telomeric DNA protects telomeres against chemotherapy-induced rapid degradation. J Mol Biol 2009, 394: 789–803.

66. Sundararaj KP, Wood RE, Ponnusamy S, Salas AM, Szulc Z, Bielawska A and Obeid LM, et al. Rapid shortening of telomere length in response to ceramide involves the inhibition of telomere binding activity of nuclear glyceraldehyde-3-phosphate dehydrogenase. J Biol Chem 2004, 279: 6152–6162.

67. Shashidharan P, Chalmers-Redman RM, Carlile GW, Rodic V, Gurvich N, Yuen T and Tatton WG, et al. Nuclear translocation of GAPDH–GFP fusion protein during apoptosis. Neuroreport 1999, 10: 1149–1153.

68. Hara MR, Agrawal N, Kim SF, Cascio MB, Fujimuro M, Ozeki Y and Takahashi M, et al. S-nitrosylated GAPDH initiates apoptotic cell death by nuclear translocation following Siah1 binding. Nat Cell Biol 2005, 7: 665–674.

69. Huang Q, Lan F, Zheng Z, Xie F, Han J, Dong L and Xie Y, et al. Akt2 kinase suppresses glyceraldehyde-3-phosphate dehydrogenase (GAPDH)-mediated apoptosis in ovarian cancer cells via phosphorylating GAPDH at threonine 237 and decreasing its nuclear translocation. J Biol Chem 2011, 286: 42211–42220.

70. Ventura M, Mateo F, Serratosa J, Salaet I, Carujo S, Bachs O and Pujol MJ. Nuclear translocation of glyceraldehyde-3-phosphate dehydrogenase is regulated by acetylation. Int J Biochem Cell Biol 2010, 42: 1672–1680.

71. Sen N, Hara MR, Kornberg MD, Cascio MB, Bae BI, Shahani N and Thomas B, *et al*. Nitric oxide-induced nuclear GAPDH activates p300/CBP and mediates apoptosis. Nat Cell Biol 2008, 10: 866–873.

72. Joo HY, Woo SR, Shen YN, Yun MY, Shin HJ, Park ER and Kim SH, *et al*. SIRT1 interacts with and protects glyceraldehyde-3-phosphate dehydrogenase

(GAPDH) from nuclear translocation: implications for cell survival after irradiation. Biochem Biophys Res Commun 2012, 424: 681–686.

73. Harada N, Yasunaga R, Higashimura Y, Yamaji R, Fujimoto K, Moss J and Inui H, *et al*. Glyceraldehyde-3-phosphate dehydrogenase enhances transcriptional activity of androgen receptor in prostate cancer cells. J Biol Chem 2007, 282: 22651–22661.

74. Heinlein CA and Chang C. Androgen receptor in prostate cancer. Endocr Rev 2004, 25: 276–308.