

# Impact of acetylation on tumor metabolism

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**Abbreviations:** aces, acetyl-coa synthetase; ACLY, ATP-citrate lyase; AMPK, AMP-activated protein kinase; CMA, chaperone-mediated autophagy; EHHADH, enoyl coa hydratase/3-hydroxylacyl coa dehydrogenase; ENO1, enolase 1; FBP, fructose-1,6-bisphosphate; FH, fumarate hydratase; G6PD, glucose-6-phosphate dehydrogenase; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GDH, glutamate dehydrogenase; GLUT1, glucose transporter isoform 1; GLS1, glutaminase; HATs, histone acetyltransferases; HDACs, histone deacetylases; HIF, hypoxia-inducible factor; IDH, isocitrate dehydrogenase; KATs, lysine acetyltransferases; LDH-A, lactate dehydrogenase; LEF/TCF, lymphoid enhancer factor/T-cell factor; MCT4, monocarboxylate transporter 4; MDH, malate dehydrogenase; NADPH, nicotinamide adenine dinucleotide phosphate; OTC, ornithine transcarbamylase; PDC, pyruvate dehydrogenase complex; PDH, pyruvate dehydrogenase; PEPCK1, phosphoenolpyruvate carboxykinase 1; PGAM, phosphoglycerate mutase; PKM2, M2 isoform of pyruvate kinase; pRB, retinoblastoma protein; PTEN, phosphatase and tensin homolog; ROS, reactive oxygen species; S1P, sphingosine 1-phosphate; SDH, succinate dehydrogenase; SIRT's, sirtuins; SOD, superoxide dismutase; SPHK, sphingosine kinase; STAT, signal transducers and activators of transcription; TPI, triosephosphate isomerase

Acetylation of protein lysine residues is a reversible and dynamic process that is controlled by histone acetyltransferases (HATs) and deacetylases (HDACs and SIRT's). Recent studies have revealed that acetylation modulates not only nuclear proteins but also cytoplasmic or mitochondrial proteins, including many metabolic enzymes. In tumors, cellular metabolism is reprogrammed to provide intermediates for biosynthesis such as nucleotides, fatty acids, and amino acids, and thereby favor the rapid proliferation of cancer cells and tumor development. An increasing number of investigations have indicated that acetylation plays an important role in tumor metabolism. Here, we summarize the substrates that are modified by acetylation, especially oncogenes, tumor suppressor genes, and enzymes that are implicated in tumor metabolism.

## Protein Acetylation System

Protein lysine acetylation is a dynamic and reversible process that is controlled by protein lysine acetyltransferases (KATs, also called histone acetyltransferases or HATs) and deacetylases (known as histone deacetylases or HDACs).

HATs are a group of enzymes that transfer the acetyl group from acetyl-CoA to lysine residues of substrate proteins. HATs were initially found to acetylate histones and decrease their

interaction with negatively charged DNA through neutralization of the positive charge of lysine residues. This process can enhance the binding of transcriptional factors with gene promoters to regulate gene expression during development, cell proliferation, and disease development. Further studies have suggested broader functions for the HAT family in the regulation of lysine acetylation of non-histone proteins, such as transcription factors and nuclear receptors, in order to control gene expression.<sup>1</sup>

HATs are conserved in evolution from yeast to human. HAT1 was the first reported histone acetyltransferase,<sup>2</sup> and to date approximately 19 members of the mammalian HAT family have been reported. On the basis of sequence homology, structural features, and functional roles, HATs are commonly grouped into several families, including p300/CBP, GNATs, MYST HATs, nuclear receptors, and co-activators.

The best-studied HATs belong to the p300/CBP subfamily. CREB-binding protein (CBP or CREBBP) and p300 increase gene expression in various ways, such as by relaxing the chromatin structure at the gene promoters through histone acetyltransferase activity or by recruiting the transcriptional machinery (e.g., RNA polymerase II) to the promoters.<sup>3</sup> These proteins bind to many sequence-specific factors involved in cell growth and differentiation, including c-jun and the adenoviral oncoprotein E1A. *CBP* or *p300* knockout mice die at an early embryonic stage, indicating that both proteins are essential for normal embryonic development.<sup>4</sup> Loss-of-function mutations in the *p300* gene have been identified in several types of cancer, including acute myeloid leukemia (AML), prostate cancer, and breast cancer.

The Gcn5-related N-acetyltransferase (GNAT) family includes GCN5, PCAF, Hat1, Elp3, Hpa2, Hpa3, ATF-2, and Nut1. These HATs are known to acetylate lysine residues on histones H2B, H3, and H4, and share a similar catalytic HAT domain. GCN5 and p300/CBP-associated factor (PCAF) are mammalian GNATs. PCAF can bind together with p300/CBP to directly regulate transcription. The targets of the acetyltransferase activity of PCAF include Fli1, p53, and numerous histone

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residues. The MYST family of HATs consists of MOZ, Ybf2 (Sas3), Sas2, Tip60, Esa1, MOF, MORF, and HBO1. These HATs acetylate lysine residues on histones H2A, H3, and H4. Tip60 was the first reported MYST family member to exhibit HAT activity in humans. Three important nuclear receptor co-activators that display HAT activity are SRC-1, ACTR, and TIF-2, all of which are known to interact with p300/CBP and PCAF. In addition to these subfamilies, several other proteins exhibit HAT activity, including TAFII250, TFIIC, Rtt109, and CLOCK.

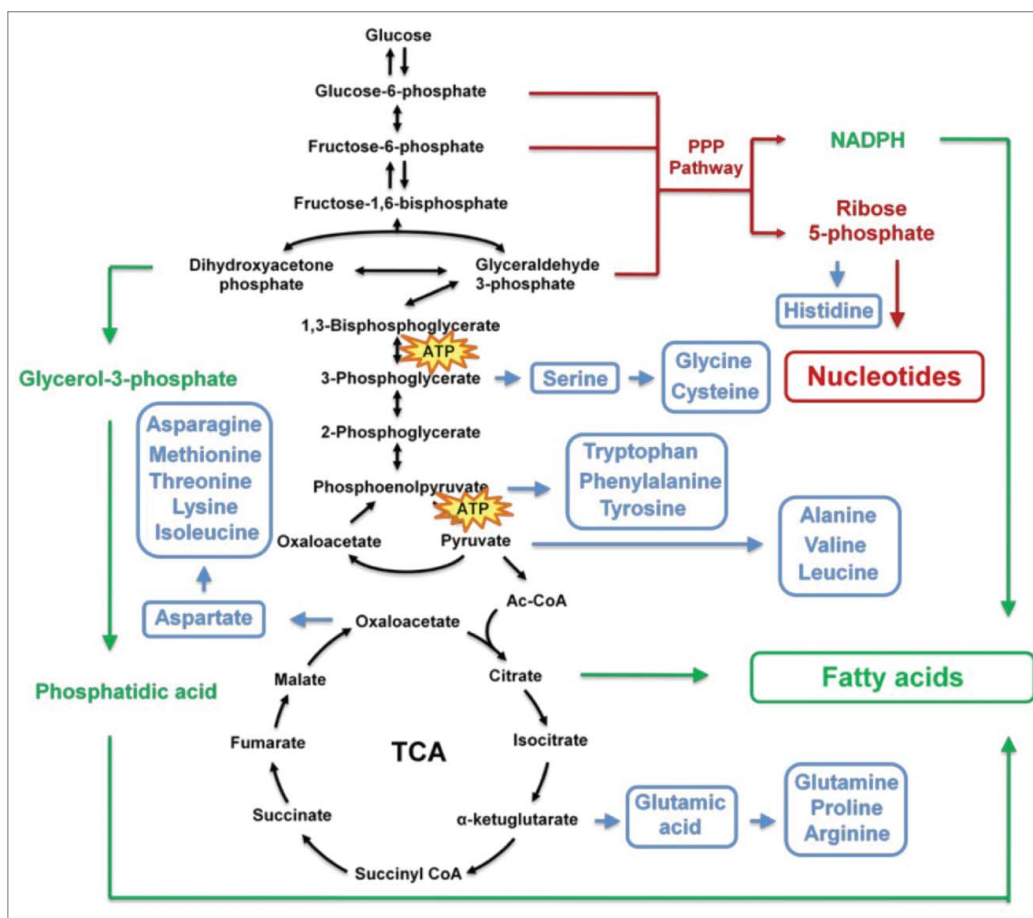
The HDACs are a group of hydrolases that remove acetyl groups from acetylated lysine residues of histone, allowing the histones to wrap the DNA more tightly to downregulate gene transcription. Although the initial HDAC substrates identified were histones, it is now clear that HDAC substrates extend far beyond these proteins. Therefore, HDACs are now also called lysine deacetylases (KDAC) to reflect their action on non-histone proteins.

HDACs are divided into 4 classes based on sequence homology and function. The first 2 classes are considered

“classical” HDACs, with activities that are inhibited by the HDAC inhibitor, trichostatin A (TSA), whereas the third class is a family of NAD<sup>+</sup>-dependent proteins (Sirtuins or SIRT) that are affected by nicotinamide (NAM) but not by TSA. The last class has one typical member, HDAC11, which shows only DNA sequence similarity to other HDACs.

Class I HDACs, including HDAC1, HDAC2, HDAC3, and HDAC8, are homologs of the yeast reduced potassium dependency 3 (Rpd3) protein and are the most ubiquitously expressed in tissues. All of these proteins localize in the nucleus, and HDAC8 also partially localizes in the cytoplasm. HDAC1 interacts with Rb protein to control cell proliferation and differentiation, and also deacetylates p53 and modulates its effect on cell growth and apoptosis.<sup>5</sup> Class II HDACs, homologs of yeast histone deacetylase 1 (hda1), comprise HDAC4, HDAC5, HDAC6, HDAC7, HDAC9, and HDAC10. HDACs in this class are able to shuttle in and out of the nucleus in response to different signals. Compared with Class I HDACs, class II HDAC proteins have relatively weak deacetylase activity and act primarily as recruiting proteins within large complexes that include class I HDACs and other regulatory elements.<sup>6</sup> Mutations in HDAC6 have been associated with Alzheimer’s disease, whereas HDAC6 overexpression correlates with tumorigenesis and cancer metastasis.<sup>7,8</sup>

SIRT, an evolutionarily conserved multigene family, are structurally distinct from the other classes of HDACs. Members of this family occupy different subcellular compartments—the nucleus (SIRT1, SIRT2, SIRT6, and SIRT7), the cytoplasm (SIRT1 and SIRT2), and the mitochondria (SIRT3, SIRT4, and SIRT5)—and participate in a wide range of cellular processes including transcription, metabolism, aging, apoptosis, inflammation, and stress resistance.<sup>9</sup> Some SIRT are also involved in tumorigenesis, control of circadian clocks, and mitochondrial biogenesis. SIRT4, SIRT5, and SIRT6 have also been found to possess other catalytic activities beyond deacetylation.<sup>10</sup>



**Figure 1.** Acetylation controls enzymes involved in tumor metabolism. Enzymes involved in different metabolism pathways, including glucose metabolism, fatty acid metabolism, and glutamine metabolism, are regulated by acetylation modification. Enzymes in the red circle are catalytically activated or exhibit increased protein stability after acetylation whereas those in the blue circle are catalytically inhibited or exhibit increased degradation after acetylation.

## Tumor Metabolism

The dysregulation of cellular metabolism is an important hallmark of cancer that was first described by Otto Warburg, which has a reciprocal causation with tumorigenesis and development. The famous “Warburg phenomenon” is an increased rate of glycolysis in cancer cells even under normal oxygen conditions (aerobic glycolysis), resulting in enhanced lactate production. Cancer cells can benefit from glycolysis through different mechanisms to promote tumor cell growth and biosynthesis of mass. Warburg also proposed that a permanent impairment of oxidative metabolism contributes to increased glycolysis, accounting for the lack of ATP generation.<sup>11</sup> However, later studies have demonstrated that tumor cells do not have defects in oxidative metabolism and most tumors retain the capacity for oxidative phosphorylation.<sup>12,13</sup> Although glycolysis in tumor cells generates ATP with low efficiency compared with normal cells, tumor cells take up much more glucose to support the high rate of glycolysis flux and produce abundant ATP for tumor survival and growth.<sup>14</sup> At the same time, the intermediate metabolites of glycolysis provide precursors for synthesis of biomacromolecules such as nucleotides, fatty acids, and nonessential amino acids to support rapid cancer cell growth (Fig. 1).

Many glycolysis intermediates of tumor cell metabolism, such as glucose-6-phosphate, fructose-6-phosphate, and glyceraldehyde 3-phosphate, can be used to produce ribose 5-phosphate and nicotinamide adenine dinucleotide phosphate (NADPH) through the pentose phosphate pathway (PPP). The generation of ribose 5-phosphate contributes to rapid nucleotide synthesis, thereby supporting DNA duplication and RNA activity during cell proliferation. Furthermore, the production of NADPH can prevent cancer cells from undergoing apoptosis by eliminating intracellular reactive oxygen species (ROS) and also provides reducing power for lipid synthesis to generate membrane structures for newly divided cells. Moreover, 3-phosphoglycerate produced in glycolysis can be used for *de novo* serine biosynthesis, feeding carbon derived from glucose into the folate cycle. The folate cycle provides purine nucleotides

for DNA and RNA synthesis and generates glutathione to maintain the NADPH/ NADP<sup>+</sup> ratio for anabolic metabolism. It has been reported that the folate cycle is activated in tumors and correlates with tumorigenesis.<sup>15</sup>

Several intermediates of glycolysis and the TCA cycle participate in fatty acid synthesis. Dihydroxyacetone phosphate produced during glycolysis can be converted into glycerol-3-phosphate, which is an important substrate for triacylglycerol synthesis by the phosphatidic acid pathway. Synthesis of fatty acids, which are used to produce cellular lipids, requires acetyl-CoA, most of which is generated from citrate transferred from the mitochondria to the cytoplasm (Fig. 1). Oxaloacetate is not only a source of citrate production for lipid synthesis, but is also an important precursor for the synthesis of many types of amino acid. In fact, many intermediates can be used for amino acid synthesis, including 3-phosphoglycerate, phosphoenolpyruvate, pyruvate, and  $\alpha$ -ketoglutarate (Fig. 1). In addition, some amino acids, such as glutamine, glycine, and aspartate, are essential for nucleotide synthesis.

These rapid biosynthesis processes result in depletion of TCA cycle intermediates. These are replenished by the process of anaplerosis, which involves 5 reactions: production of oxaloacetate from pyruvate catalyzed by pyruvate carboxylase; reversible conversion of aspartate to oxaloacetate catalyzed by aspartate transaminase; generation of  $\alpha$ -ketoglutarate from glutamate catalyzed by glutamate dehydrogenase; formation of succinyl-CoA by odd-chain fatty acid oxidation; and the generation of fumarate from adenylosuccinate.<sup>16</sup> In addition to glycolysis, glutaminolysis is another major source of energy production in cancer cells. Tumor cells use glutamine to generate glutamate and then produce  $\alpha$ -ketoglutarate for the remaining TCA cycle steps from  $\alpha$ -ketoglutarate to citrate. A high extracellular glutamine concentration not only promotes tumor growth but is also essential for cell transformation. Furthermore, some cancer types display addiction to glutamine, such as pancreatic cancer, glioblastoma, acute myelogenous leukemia, and small-cell lung cancer. The sensitivity of such cancers to glutamine starvation provides a new therapeutic target in cancer treatment.<sup>17</sup>

**Table 1.** Acetylation of oncogenes and tumor suppressor genes

Name	Related tumors	Acetylation site	HATs	HDACs	Acetylation effects
HIF-1	Renal cell cancer, breast cancer	K674	PCAF <sup>42</sup>	SIRT1 <sup>43</sup>	Delays HIF-1 $\alpha$ degradation to stimulate anaerobic glycolysis
Myc	Lymphoma, breast cancer	K323, K417	P300 <sup>46</sup> ; CBP <sup>45</sup> ; GCN5/PCAF/TIP60 <sup>47</sup>	—	Stimulates oncogene expression
KRAS	Lung carcinoma, pancreatic cancer, colon cancer	K104	—	HDAC6 <sup>49</sup> SIRT2 <sup>49</sup>	Attenuates transforming activity and suppresses tumor cell growth
p53	Pancreatic cancer, breast cancer, lung cancer	K305, K320, K370, K372, K373, K381, K382	p300/CBP <sup>54</sup> ; PCAF <sup>54</sup>	SIRT1	Promotes DNA-binding ability; modulates stability
pRb	Breast cancer, prostate cancer	K873, K874	p300/CBP <sup>62</sup>	HDAC1	Blocks its phosphorylation by CDKs; retains pRb in an active state of growth suppression
PTEN	Glioblastoma, breast cancer, lung cancer, prostate cancer	K125, K128, K402	CBP <sup>57</sup> ; PCAF <sup>58</sup>	SIRT1 <sup>57</sup>	Promotes interaction with PDZ domain-containing proteins; interferes with catalytic specificity toward PIP3

During tumorigenesis and development, the cells undergo metabolic reprogramming such that energy production is redirected to balance energy needs and macromolecular biosynthesis, ultimately promoting cell growth. A principal mechanism of metabolic reprogramming in cancer cells is alteration of metabolic enzyme systems. Hypoxia-inducible factor (HIF) is a transcription factor that is primarily activated by hypoxic stress.<sup>18</sup> The activation of HIF in tumors can stimulate the conversion of glucose to pyruvate and lactate by upregulation of glucose transporter isoform 1 (GLUT1), hexokinase, monocarboxylate transporter 4 (MCT4), and lactate dehydrogenase A.<sup>19</sup> Moreover, HIF-1 also decreases the conversion of pyruvate to acetyl-CoA by upregulating PDH kinase 1 (PDK1) to inhibit pyruvate dehydrogenase (PDH).<sup>20,21</sup> Activation of oncogenes (e.g., Myc, Ras) and inactivation of tumor suppressors (e.g. PTEN, P53) also contribute to the regulation of metabolic enzyme transcription during cancer cell reprogramming.<sup>22,23</sup> On the other hand, loss-of-function mutations of fumarate hydratase (FH), succinate dehydrogenase (SDH), and isocitrate dehydrogenase (IDH), which have been reported in different tumors, result in accumulation of the TCA cycle intermediates fumarate, succinate, and 2-hydroxyglutarate respectively. These metabolites competitively inhibit the  $\alpha$ -ketoglutarate-dependent dioxygenases that regulate epigenetic mechanisms and tumorigenesis.<sup>24,25</sup> In this regard, fumarate, succinate, and 2-hydroxyglutarate are also referred to as oncometabolites. This serves as a good example of the notion that metabolic enzymes can be important oncogenic players.

With the development of improved technology for studying tumor biology, the dysregulation of cellular metabolism that occurs before tumorigenesis and progression has gradually been validated by experiments. In 2009, Yun and colleagues found that a lack of glucose promotes *KRAS* WT cells to gain *KRAS* and *BRAF* mutations; this was the first study to demonstrate that dysregulation of cellular metabolism leads to tumorigenesis.<sup>26</sup> Subsequently, C-labeled pyruvate molecular imaging technology showed that alterations in glycolysis occur before *c-Myc* induces tumor formation and shrinkage *in vivo*.<sup>13</sup> In addition, the accumulation of oncometabolites such as fumarate, succinate, and 2-hydroxyglutarate competitively inhibits the  $\alpha$ -ketoglutarate-dependent dioxygenases and plays an important role in tumorigenesis.<sup>24,25</sup> These studies demonstrate that dysregulation of cellular metabolism plays an initial role in tumorigenesis. These data also suggest a novel approach to antitumor therapy by directly targeting tumor metabolic pathways.

Indirect targets consist of upstream regulators and proteins involved in signaling pathways of metabolism, such as the restoration of apoptosis, interruption of cell-autonomous growth signals, and inhibition of angiogenesis; such proteins include HIF, PI3K, AKT, mTOR, and AMP-activated protein kinase (AMPK).<sup>27-29</sup> Studies also proved that some enzymes that are directly involved in cancer metabolism could confer antitumor therapeutic vulnerabilities. In glioblastoma, deletion of the glycolytic gene enolase 1 (*ENO1*) in tumor cells is tolerated through the expression of *ENO2*. Muller et al. demonstrated that selective silencing of *ENO2* by shRNA inhibits growth, survival, and the tumorigenic potential of *ENO1*-deleted GBM cells and increases

the sensitivity of these cells to enolase inhibitors.<sup>30</sup> More common approaches include suppressing the enzymes involved in tumor reprogramming or targeting tumor-specific enzyme isoforms. For example, 3-bromopyruvate inhibits HK2 and results in apoptosis in hepatocellular carcinomas,<sup>23</sup> inhibition of PDK1 restores PDH activity and triggers apoptosis in tumor cells,<sup>31</sup> LDH-A inhibition induces oxidative stress and inhibits tumor progression,<sup>32</sup> and targeted inhibition of mutant IDH1 delays the growth of glioma.<sup>33</sup> These therapeutic methods not only limit tumor-specific bioenergetic flow and anabolic reactions, but also reverse the neoplastic phenotype.

## Histone Acetylation and Tumor Metabolism

Histone acetylation regulates chromatin modification and affects the expression of numerous genes implicated in oncogenesis. Altered expression and mutations of class I or II HDAC genes have been linked to tumor development through induction of aberrant transcription of key genes regulating cell proliferation, cell cycle transition, differentiation, and apoptosis.<sup>34</sup> Moreover, HDAC inhibitors were shown to reverse the transformed cell phenotype and have been considered as promising cancer therapeutics.<sup>35,36</sup> These studies indicated the important roles of histone acetylation in tumor metabolism.

Metabolism intermediates such as butyrate, acetyl-CoA, and acetate have been reported to influence histone acetylation. Before 1977, n-butyrate had been shown to increase histone acetylation in neuroblastoma, HeLa, and other tumor cells, thereby inhibiting cell proliferation and affecting DNA content and cell morphology.<sup>37</sup> Acetyl-CoA is a broad substrate for histone acetylation. Recently, Lee et al. demonstrated that changes in acetyl-CoA availability mediated by oncogenic metabolic reprogramming contribute to histone acetylation levels in tumor cells.<sup>38</sup> They also found that oncogenic *KRAS* or *AKT* stimulates histone acetylation through the metabolic enzyme ATP-citrate lyase (ACLY). *AKT*-dependent alterations in nuclear acetyl-CoA level modulate global histone acetylation levels preceding tumor development in gliomas and prostate cancers. These results implicate acetyl-CoA metabolism as a key regulator of histone acetylation in cancer cells.<sup>38</sup> Evidence that acetyl-CoA controls HAT activity comes from findings that depletion of acetyl-CoA reduced the activity of p300 and impaired its suppressive effect on autophagy.<sup>39</sup> Interestingly, Thompson's lab found that ACLY, which converts glucose-derived citrate into acetyl-CoA, is required for the increase in histone acetylation in response to growth factor stimulation and during differentiation.<sup>40</sup> This study also reported that ACLY translocates into the nucleus and generates acetyl-CoA to induce histone acetylation and modulate expression of glycolysis genes in tumor metabolism. In addition to ACLY, pyruvate dehydrogenase complex (PDC) also contributes to acetyl-CoA generation in the nucleus of mammalian cells. A recent elegant investigation showed that knockdown of nuclear PDC decreased acetyl-CoA synthesis and acetylation of core histones<sup>41</sup>. These authors also found that PDC translocates from mitochondria to the nucleus in a cell cycle-dependent manner and provides

a mechanism of nuclear acetyl-CoA synthesis for histone acetylation and epigenetic regulation.<sup>41</sup>

## Regulation of Oncogenes and Tumor Suppressor Genes by Acetylation

Many oncogenes and tumor suppressor genes have been linked to cancer metabolism. Intensive studies have found that this process is heavily regulated by acetylation, indicating that acetylation of these proteins contributes to functions implicated in tumor metabolism. These findings are summarized below.

### Oncogenes

As mentioned previously, hypoxia-inducible factor (HIF-1) plays a critical role in the regulation of tumor metabolism by mediating the switch in energy metabolism from oxidative phosphorylation to anaerobic glycolysis during hypoxic adaptation and tumor metabolism. To date, more than 80 genes (including most of the glycolytic enzymes) have been found to be regulated by HIF-1. HIF-1 is a heterodimer composed of HIF-1 $\alpha$  and HIF-1 $\beta$ . The stability and activity of HIF-1 are predominantly determined by HIF-1 $\alpha$ . It has been reported that PCAF acetylates HIF-1 $\alpha$  at K674, which delays its degradation.<sup>42</sup> Lim et al. found that SIRT1 deacetylates and inactivates HIF-1 $\alpha$  by blocking p300 recruitment, leading to repression of HIF-1 target genes. Under hypoxia, SIRT1 is downregulated to allow the activation of HIF-1 $\alpha$  and stimulate anaerobic glycolysis. These authors also showed that SIRT1 deacetylates HIF-1 $\alpha$  and suppresses target gene transcription, thereby inhibiting tumor metabolism and tumor growth.<sup>43</sup>

Myc functions as a transcription factor and binds to 10–15% of all promoter regions. By modifying expression of its target genes, Myc activation plays an important role in numerous biological effects including cell proliferation, apoptosis, differentiation, and stem cell self-renewal. Myc also contributes to the metabolic reprogramming of tumor cells at multiple levels. Myc not only activates expression of the glucose transporter GLUT1, the lactate transporter MCT1, and lactate dehydrogenase (LDH-A) to promote the Warburg effect, but also prevents pyruvate entering the TCA cycle by upregulating PDK. Moreover, Myc stimulates glutamine transporters but inhibits glutaminase (GLS1) to support the glutamine addiction of cancer cells. In addition, Myc activates genes involved in ribosomal and mitochondrial biogenesis, nucleotide synthesis, and lipid synthesis.<sup>44</sup> Myc is a strong proto-oncogene and is mutated in many types of cancers. In 2003, Vervoorts et al. reported that Myc is acetylated by CBP.<sup>45</sup> Subsequent studies demonstrated that Myc could be also a substrate of the acetyltransferases P300, GCN5, PCAF, and TIP60.<sup>46,47</sup> Acetylation was found to inhibit ubiquitin-mediated degradation of Myc and thereby stabilize Myc protein. However, some reports suggested dual roles of p300/CBP in Myc regulation: acting as a coactivator it stabilizes Myc whereas acting as an acetylase it destabilizes Myc.<sup>46</sup> Further studies are

needed to clarify how acetylation affects Myc, in particular its functions in tumor metabolism.

Normal KRAS performs an essential function in cellular signaling, and activating mutations of KRAS are critical in the development of many cancers, such as lung adenocarcinoma, pancreatic ductal carcinoma, and colorectal carcinoma. Recently, researchers found that KRAS is acetylated at K104. This acetylation attenuates KRAS transforming activity by interfering with GEF-induced nucleotide exchange.<sup>48</sup> Later, the same group reported that the deacetylases HDAC6 and SIRT2 could regulate the acetylation of KRAS in cancer cells. Inhibition of these deacetylases suppresses the growth of cancer cells that express activated mutants of KRAS.<sup>49</sup> In 2012, Ying et al. revealed that oncogenic KRAS could regulate anabolic glucose metabolism to maintain pancreatic tumors. Transcriptome and metabolome analysis indicated that KRAS (G12D) increases glucose uptake, enhances metabolism of glucose intermediates in the hexosamine biosynthesis and pentose phosphate pathways (PPP), and promotes ribose biogenesis.<sup>50</sup> Further study may reveal the effects of KRAS acetylation on tumor metabolism.

### Tumor Suppressor Genes

Upon DNA damage, the tumor suppressor p53 can activate DNA repair proteins, arrest the cell cycle at the G1/S checkpoint, and initiate apoptosis. Many studies have investigated a genetic link between TP53 variation and various cancers, such as pancreatic cancer, breast cancer, and lung cancer. P53 controls central carbohydrate metabolism (glycolysis, oxidative phosphorylation, and pentose phosphate pathways), and also regulates lipid metabolism. P53 is tightly associated with cancer metabolism through TIGAR, SCO2, and PGM.<sup>51–53</sup> Notably, acetylation plays an important role in p53 function. The acetylation of p53 by p300/CBP at K305, K370, K372, K373, K381, and K382 or by PCAF at K320 increases its DNA-binding ability and activates the transcription of p53 target genes.<sup>34</sup> Another study mentioned the role of site-specific p53 acetylation in transcriptional regulation induced by different types of damage. SIRT1 was shown to deacetylate p53 at K382 and inhibit its activity. Later, Chao et al. found that abrogation of acetylation of the K320 homolog in mice enhances p53-mediated apoptosis after DNA damage.<sup>54</sup> Acetylation also modulates the stability of p53 because many acetylated lysines are also sites of ubiquitylation. Acetylation can therefore protect p53 from ubiquitin-proteasome degradation.

Phosphatase and tensin homolog (PTEN) is a major tumor suppressor gene that is mutated at high frequency in many cancers, including glioblastoma, breast cancer, lung cancer, and prostate cancer. Up to 70% of patients with prostate cancer have lost a copy of the PTEN gene at the time of diagnosis.<sup>55</sup> PTEN promotes oxidative phosphorylation and decreases glycolysis. During tumor development, PTEN mutations lead to metabolic reprogramming in cancer cells and thus increase cell proliferation.<sup>56</sup> In 2008, PTEN was reported to be acetylated at K402. This study showed that CBP acetylates PTEN, whereas SIRT1 is mainly responsible for PTEN deacetylation. K402 acetylation modulates

PTEN interaction with PDZ domain-containing proteins, indicating its regulatory function.<sup>57</sup> Four years later, another group reported that PCAF interacts with and acetylates PTEN at lysines 125/128; this downregulates PTEN activity by interfering with its catalytic specificity toward PIP3.<sup>58</sup> The PI3K/AKT/mTOR pathway is a major regulator of aerobic glycolysis and cellular biosynthesis. In contrast, activation of the PI3K system is tightly controlled by the dephosphorylation of phosphatidylinositol species by PTEN. Recently, ENTPD5, a newly discovered component of the PTEN pathway, was found to promote the Warburg effect.<sup>59</sup> These studies indicate that acetylation of PTEN may play an important role in tumor metabolism.

The retinoblastoma protein (pRb) is a tumor suppressor that prevents excessive cell growth by inhibiting the cell cycle. Inactivation of pRb can lead to many types of cancer. Recent studies have linked pRb to cell metabolism through regulation of glucose tolerance, mitogenesis, and glutathione synthesis.<sup>60</sup> Moreover, pRb controls the expression of genes involved in central carbon metabolism, lipid metabolism, and nucleotide metabolism.<sup>61</sup> It has been reported that adenovirus E1A stimulates the acetylation of pRb by p300 and participates in cell cycle control. Acetylation at K873/874 also blocks the phosphorylation of pRb by cyclin-dependent kinases and thereby retains pRb in an active state of growth suppression.<sup>62</sup> A subsequent study found that pRb acetylation also plays a role in the DNA damage signaling pathway.<sup>63</sup>

## Control of Metabolic Enzymes by Acetylation

To date, 4 important acetylation proteomic studies have been reported. Together, they identified more than 4,000 potentially acetylated proteins in different cell lines and organ tissues.<sup>64-67</sup> These studies have made tremendous contribution to the field of acetylation, especially as most of the potential acetylated proteins identified are non-histone proteins. Notably, nearly all of the enzymes involved in glycolysis, gluconeogenesis, TCA cycle, fatty acid  $\beta$ -oxidation, urea cycle, nitrogen metabolism, and glycogen metabolism are acetylated. More importantly, further studies demonstrate that protein acetylation plays an essential role in metabolism, directing attention away from modification by phosphorylation and ubiquitylation.

Interestingly, acetylation modulates different metabolic enzymes in different manners. Acetylation controls the catalytic activity of enzymes through neutralization of the lysine residue, thus changing the conformation of the active site, antagonizing allosteric activation, blocking substrate interaction, or affecting the structural polymerization state.<sup>68</sup> For example, ornithine transcarbamylase (OTC) is acetylated at K88, which is located in the active site for substrate binding. Acetylation most likely inhibits OTC catalytic activity by neutralizing the positive charge of K88 and reducing substrate binding.<sup>69</sup>

Acetylation could also affect enzyme degradation through the ubiquitin-proteasomal pathway or the lysosomal pathway. Crosstalk between acetylation and ubiquitylation contributes to acetylation-mediated proteasomal degradation of enzymes. In general,

as acetyl groups and ubiquitin compete for the same lysine residues, acetylation protects enzymes from ubiquitin-proteasomal degradation; for example, acetylation of ACLY blocks its degradation.<sup>70</sup> Interestingly, acetylation of phosphoenolpyruvate carboxykinase 1 (PEPCK1) stimulates its interaction with the E3 ubiquitin ligase UBR5 to promote PEPCK1 ubiquitylation and proteasomal degradation.<sup>70</sup> In addition, acetylation regulates lysosomal degradation of enzymes by promoting interaction with chaperones, such as HSC70, that target enzyme transport into the lysosome for degradation. Recently we revealed that acetylation promotes lysosomal degradation of PKM2 and LDHA via chaperone-mediated autophagy.<sup>71,72</sup>

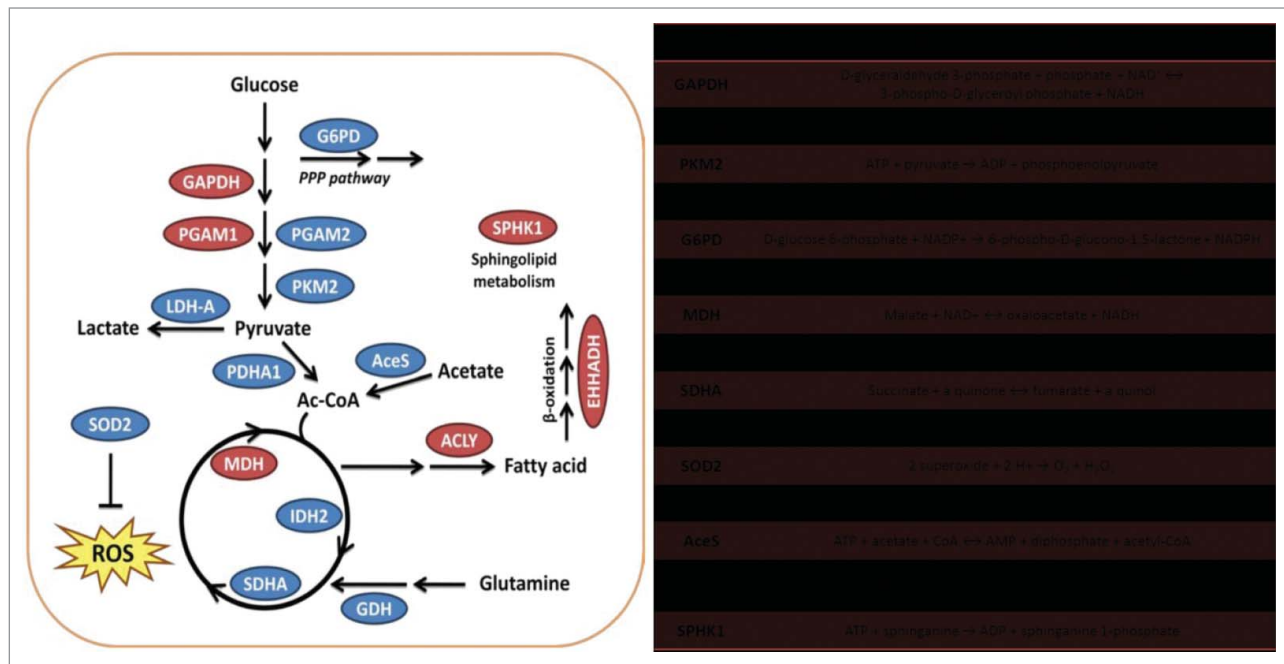
In addition, acetylation could regulate enzymes in other ways, such as through altered subcellular localization or catalytic reaction. There is emerging evidence that lysine acetylation of metabolic enzymes links signaling pathways to metabolism pathways and plays an important role in cancer cells. Many enzymes that are modulated by acetylation have been reported; in this review we discuss only the acetylated enzymes that play critical roles in tumor metabolism (Fig. 2).

## Glucose Metabolism

### Glycolysis pathway

In glycolysis and gluconeogenesis, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) catalyzes a reversible reaction between glyceraldehyde 3-phosphate and 1, 3-bisphosphoglycerate. It has been reported that acetylation regulates GAPDH glycolytic activity in *Salmonella*. Acetylation of GAPDH responds well to different signals such as glucose and acetate, and adjusts the direction of carbon flux according to environmental nutrient changes.<sup>73</sup> Recently, we reported a new acetylation site of GAPDH at K254, and showed that a high glucose concentration increases its acetylation and catalytic activity. We also demonstrated the role of GAPDH acetylation at K254 in accelerating tumor growth.<sup>74</sup>

Mammalian phosphoglycerate mutase (PGAM) catalyzes the reversible conversion of 3-phosphoglycerate (3-PGA) to 2-phosphoglycerate (2-PGA) in glycolysis. PGAM contributes to energy production through the generation of reducing power and the biosynthesis of nucleotide precursors and amino acids. Inhibition of PGAM by RNA interference or small molecules induces cell proliferation and tumor growth, and PGAM activity is commonly upregulated in tumor cells. These findings indicate an important role of PGAM in tumor metabolism. Both isoforms of PGAM (PGAM1 and PGAM2) have been reported to be acetylated. Acetylated PGAM1 displays enhanced activity, thereby stimulating glycolysis flux, and is deacetylated by SIRT1. Glucose restriction leads to deacetylation and attenuated activity of PGAM1 by increasing Sirt1 levels.<sup>75</sup> Another report found that PGAM2 is acetylated at K100, an active site residue that is invariably conserved from bacteria to mammals. Acetylation of K100 of PGAM2 decreases its activity, and SIRT2 is also the deacetylase for this isoform. Reactive oxygen species (ROS) promote the interaction of PGAM2 with SIRT2, resulting in deacetylation and activation of PGAM2. Moreover, K100 acetylation reduced



**Figure 2.** Tumor metabolism provides precursors for biomacromolecule synthesis. Metabolism in tumor cells is reprogrammed compared to that in normal cells. Upregulation of glycolysis and altered TCA flow produces intermediates that provide precursors for macromolecule biosynthesis, such as nucleotides (pathway in red), fatty acids (green), and amino acids (blue). These alterations in tumor cells support rapid cancer cell growth.

cellular NADPH production and inhibited cell proliferation and tumor growth.<sup>74</sup>

In the last 5 years, intensive studies have focused on the M2 isoform of pyruvate kinase (PKM2). PKM2 catalyzes the last step in glycolysis, from phosphoenolpyruvate to pyruvate, and is highly expressed in embryonic tissues and many cancer types. Alternative splicing of the *PKM2* gene produces 2 isoforms, M1 and M2. Expression of the inactive PKM2 isoform causes rapidly dividing cells to accumulate glycolytic metabolites that drive macromolecular biosynthesis and cell growth. In 2011, we found that acetylation at K305 decreases PKM2 enzyme activity and promotes its lysosomal-dependent degradation. High glucose concentrations stimulate PKM2 acetylation by increasing its binding to the acetyltransferase PCAF. We also demonstrated that K305 acetylation leads to accumulation of glycolytic intermediates to promote cell proliferation and tumor growth.<sup>71</sup>

In tumor cells, lactate is generated from pyruvate by lactate dehydrogenase A (LDH-A), which is frequently overexpressed in tumor cells and is important for cell growth. LDH-A regenerates NAD<sup>+</sup> for rapid glycolysis in tumor cells in addition to the production of lactate, giving rise to an acidic microenvironment that promotes tumor cell migration and metastasis. Recently, we reported that the acetylation of LDH-A at K5 inhibits its activity and targets LDH-A degradation by chaperone-mediated autophagy. We also showed that LDH-A acetylation decreases cell migration and tumor growth. Moreover, reduced K5 acetylation during the initiation of human pancreatic cancers contributes to accumulation of LDH-A and promotes tumor metabolism and cancer progression.<sup>72</sup>

### TCA cycle pathway

The pyruvate dehydrogenase complex (PDC), which plays an important role in glucose homeostasis, is negatively regulated through phosphorylation of pyruvate dehydrogenase (PDH) by PDH kinase (PDK) and activated by PDH phosphatase (PDP). A recent study reported that PDHA1 and PDP1 are acetylated in EGF-stimulated cells and diverse human cancer cells, and ACAT1 and SIRT3 were identified as the acetyltransferase and deacetylase responsible. K321 acetylation inhibits PDHA1 through recruitment of PDK1, whereas PDP1 acetylation at K202 decreases its interaction with PDHA1, leading to increased cancer cell glycolysis and tumor growth.<sup>76</sup>

Several enzymes in the TCA cycle have been reported to be acetylated. The activity of mitochondrial isocitrate dehydrogenase 2 (IDH2), which catalyzes the decarboxylation of isocitrate to  $\alpha$ -ketoglutarate, is inhibited by acetylation. Under caloric restriction, IDH2 is deacetylated and activated by SIRT3 to protect against oxidative damage by increasing NADPH production.<sup>77</sup> Malate dehydrogenase (MDH) is also acetylated at K185, K301, K307, and K314 in response to high glucose.<sup>64</sup> Succinate dehydrogenase (SDH) is a unique enzyme that participates in both the TCA cycle and in oxidative phosphorylation (as complex II). Deacetylation of one of its subunits, SDHA, by SIRT3 is accompanied by the activation of SDHA complex II activity.<sup>78</sup> By mass spectrometry, 4 acetylated lysine residues were found to be located on the hydrophilic surface of SDHA, indicating that acetylation may hinder the entry of substrates into the active site.<sup>68</sup> So far, acetylation of these enzymes has not been linked to tumors; however, the products produced by activity of these

enzymes, such as  $\alpha$ -ketoglutarate and succinate, have been reported to modulate epigenetics in some cancer types. These findings indicate the potential functions of acetylation of TCA cycle enzymes in tumor metabolism.

### Pentose phosphate pathway (PPP)

The PPP is controlled by glucose-6-phosphate dehydrogenase (G6PD), which provides reducing power and ribose phosphate for nucleotide and lipid biosynthesis and redox balance. There is ample clinical evidence that G6PD is frequently overexpressed in multiple myeloma, chronic lymphocytic leukemia, and gastric cancer.<sup>79</sup> G6PD has been revealed as a promising target in cancer therapy.<sup>80</sup> A recent study reported that G6PD is negatively regulated by acetylation on K403. This acetylation inhibits formation of the active dimer and causes a complete loss of catalytic activity. Knockdown of *G6PD* sensitizes cells to oxidative stress, and re-expression of a K403 acetylation mimetic mutant impairs the rescue of cells from oxidative injury. Moreover, SIRT2-mediated deacetylation and activation of G6PD stimulates the PPP to supply NADPH in order to resist oxidative damage and protect mouse erythrocytes. KAT9/ELP3 was identified as a potential acetyltransferase of G6PD.<sup>74</sup> This study suggested the potential role of G6PD acetylation in tumor metabolism.

### Lipid Metabolism

ATP-citrate lyase (ACLY) is an enzyme involved in fatty acid biosynthesis that links carbohydrate metabolism and fatty acid production. ACLY is responsible for the synthesis of cytosolic acetyl-CoA in many tissues. ACLY is upregulated or activated in various types of cancer, and inhibition of ACLY represses cancer cell proliferation. Recently, we found that ACLY is acetylated at K540, K546, and K554. PCAF and SIRT2 are responsible for regulating ACLY acetylation. High glucose stimulates acetylation and increases ACLY stability by blocking its ubiquitylation and degradation. Substitution of these 3 lysine residues stabilizes ACLY and promotes *de novo* lipid synthesis, cell proliferation, and tumor growth. Moreover, acetylation of ACLY is increased in human lung cancers.<sup>81</sup>

Microarray-Bioinformatic analysis revealed that many genes involved in fatty acid metabolic pathways are overexpressed in colorectal cancer, including the gene encoding enoyl CoA hydratase/3-hydroxyacyl CoA dehydrogenase (EHHADH).<sup>82</sup> EHHADH is the key enzyme in fatty acid  $\beta$ -oxidation and contains 2 domains: enoyl-CoA hydratase (N-terminal) and 3-hydroxyacyl-CoA dehydrogenase (C-terminal). Recently, Zhao et al. reported that EHHADH is modified by acetylation at 4 lysine residues (K165, K171, K346, and K584), which increases its enzymatic activity by up to 80%. Fatty acids in the culture medium stimulate the acetylation and activation of EHHADH.<sup>64</sup>

Sphingosine kinase (SPHK), an oncogenic lipid kinase, regulates the sphingolipid metabolic pathway. Its 2 isoforms (SPHK1 and SPHK2) catalyze the conversion of D-erythro-sphingosine to sphingosine 1-phosphate (S1P), which is a signaling molecule involved in cell proliferation and survival through G protein-coupled receptor signaling. Accumulation

of S1P is linked to the progression of various cancers, thus inhibitors of SPHK represent a novel class of compounds that have potential as anticancer agents.<sup>83</sup> Some studies have demonstrated that SPHK1 is acetylated by p300 or CBP, and that this acetylation regulates protein stabilization. Acetylation and ubiquitylation compete for the same lysine residues and therefore acetylation inhibits SPHK1 ubiquitylation and degradation.<sup>84</sup> These data suggest that SPK1 acetylation plays a key role in cell growth and tumor metabolism.

### Glutamine Metabolism

In order to replenish oxaloacetate in the TCA cycle, many cancer cells require elevated levels of glutamine. Glutamate dehydrogenase (GDH) catalyzes the reversible conversion of glutamate to  $\alpha$ -ketoglutarate and ammonium in the truncated TCA. Indeed, inhibition of GDH activity sensitizes glioblastoma cells to glucose deprivation. GDH has been reported to be acetylated, and calorie restriction stimulates its acetylation in response to different nutrient conditions. *In vitro* deacetylation of GDH by SIRT3 activates the enzyme, and studies in *Sirt3* knockout mice indicated that GDH is a prominent acetylated protein in the liver.<sup>85,86</sup> SIRT4, which is localized in the mitochondria, has NAD<sup>+</sup>-dependent ADP-ribosylase activity but not NAD<sup>+</sup>-dependent deacetylase activity.<sup>87</sup> GDH is ADP-ribosylated by SIRT4 *in vitro* and this modification appears to inhibit GDH activity. Moreover, GDH activity is significantly decreased in *Sirt4*-deficient cells. These studies have established the complex regulation of GDH through different post-translational modifications by SIRT3 and SIRT4.

### Acetate Metabolism

Acetyl-CoA synthetase (AceS) was the first enzyme shown to be acetylated. AceS catalyzes the transformation of acetate into acetyl-CoA, which enters the TCA cycle for energy production or the glycolysis pathway for intermediate biosynthesis. AceS is essential for growth of bacteria on acetate or propionate as carbon sources. Acetylation of AceS of *S. enterica* at K609 is essential for AceS catalysis.<sup>88</sup> Acetylation strongly inhibits AceS activity both *in vitro* and *in vivo*. AceS purified from a CobB (SIRT homolog in *S. enterica*) mutant strain shows significantly reduced activity compared to that purified from wild-type cells, leading to poor growth of CobB mutant cells on propionate or acetate medium. Several studies have also reported that the 2 mammalian AceS isoforms (AceS1 in cytoplasm and AceS2 in mitochondria) are similarly regulated by acetylation. PAT, a bacterial protein acetyltransferase, acetylates AceS1 *in vitro* primarily at K661, which is conserved with K609 in *S. enterica*, thus inactivating the enzyme. *In vivo* experiments have found that acetylated AceS1 can be deacetylated by SIRT1, resulting in activation.<sup>89</sup> Another investigation demonstrated that mitochondrial AceS2 is also acetylated by PAT *in vitro* and deacetylated by SIRT3. Acetylation similarly inhibits AceS2 catalytic activity.<sup>90</sup> In mammalian cells, the cytosolic AceS1 produces acetyl-CoA to provide a critical substrate for biosynthesis, such



as fatty acid synthesis, whereas mitochondrial AceS2 generates acetyl-CoA for cellular energy production through the TCA cycle. Studies have found that acetate is used as a carbon source in tumor metabolism to support rapid tumor growth, indicating that regulation of AceS1 and AceS2 by acetylation may play an important role in tumor metabolism.<sup>91</sup>

## ROS Metabolism

An early hypothesis suggested that the tumor suppressive role of SIRT3 comes from protection against cellular ROS levels, and is supported by a series of studies reporting that superoxide dismutase (SOD) is a substrate of SIRT3. SOD catalyzes the dismutation of superoxide ( $O_2^{\cdot-}$ ) into oxygen and hydrogen peroxide, and is therefore an important antioxidant defense for cells that are exposed to oxygen. Superoxide is quite toxic *in vivo*, and knockout of *SOD* not only shortens individual lifespan, but also accelerates certain features of aging. Deficiency of SOD is linked to various human diseases including cancers. Three investigations found that acetylation inhibits SOD2 catalytic activity, whereas oxidative stress-induced deacetylation by SIRT3 results in SOD2 activation and ROS reduction; however, these studies identified different acetylated lysine residues in SOD2.<sup>92-94</sup>

## Future Prospects

A number of studies have recently established the critical role of acetylation in the regulation of metabolism, especially cancer metabolism. However, despite the rapid progress in this field many important questions remain largely unanswered.

## Non-Metabolic Functions of Metabolic Enzymes

Although the enzymes discussed above are commonly responsible for tumor metabolism, recent studies have revealed that some of them have non-metabolic functions in cancer.

Recent studies have proven that PKM2 has non-metabolic functions in the nucleus as a protein kinase and a transcriptional cofactor. Luo et al. reported that PKM2 interacts with HIF-1 $\alpha$  in the nucleus to promote the transactivation of HIF-1 target genes for the reprogramming of glucose metabolism in cancer cells.<sup>95</sup> However, Yang et al. reported that EGFR induces nuclear localization of PKM2 to promote its interaction with  $\beta$ -catenin and thereby stimulate cyclin D1 expression.<sup>96</sup> Gao et al. reported that homodimers of PKM2 could phosphorylate STAT3.<sup>97</sup> More recently, PKM2 was found to directly phosphorylate histone H3 upon EGFR activation, leading to the activation of oncogenes.<sup>98</sup> Interestingly, we found that PKM2 is acetylated by p300 at K433, which is unique to PKM2 and directly contacts its allosteric activator, fructose-1,6-bisphosphate (FBP). Acetylation prevents PKM2 activation by interfering with FBP binding and promotes PKM2 nuclear accumulation and its protein kinase activity, leading to cell proliferation and tumorigenesis.<sup>99</sup>

Several studies have found that GAPDH is not only a classic metabolic protein, but also plays important roles in a variety of critical nuclear pathways, including apoptosis, transcriptional regulation, nuclear membrane fusion, and the maintenance of telomere structure.<sup>100</sup> The translocation of GAPDH into the nucleus can be influenced by cellular growth conditions and is regulated by acetylation. Mammalian GAPDH is acetylated by PCAF, leading to GAPDH nuclear translocation, and treatment of cells with the HDAC inhibitor TSA promotes GAPDH nuclear accumulation.<sup>101</sup>

Another example is triosephosphate isomerase (TPI), which converts dihydroxyacetone phosphate into glyceraldehyde-3-phosphate in glycolysis. TPI deficiency leads to serious neurological symptoms. Roland et al. found that the *Drosophila* TPI sugarkill mutant, which closely models TPI deficiency, can be genetically complemented by catalytically inactive TPI; this indicates a non-metabolic function of TPI, loss of which contributes to neurological dysfunction.<sup>102</sup>

These studies suggest that the regulatory roles of acetylation on metabolic enzymes go beyond their classic catalytic functions in human diseases. We expect that more of these enzymes will be shown to have non-metabolic functions in the future.

## Multiple Signaling Pathways Modulated by Acetylation

Acetylation is an important regulator of histones, transcriptional factors, and metabolic enzymes. In addition, many other processes are modulated by acetylation, such as mRNA splicing, mRNA transport, mRNA integrity, and protein translation. Multiple signaling pathways in cancer are regulated by acetylation, but due to space limitations we will discuss only the NF- $\kappa$ B pathway, Wnt pathway, and EGFR signaling pathway.

As a regulator of gene transcription, NF- $\kappa$ B controls cell proliferation and cell survival. Many types of tumor have been shown to have dysregulated NF- $\kappa$ B. The activity of NF- $\kappa$ B is tightly regulated by acetylation. NF- $\kappa$ B is a heterodimer of RelA/p65 and p50/p52 subunits. In resting cells, NF- $\kappa$ B is associated with its repressor and localized in the cytoplasm. Upon stimulation, the repressor is inhibited and the released NF- $\kappa$ B translocates to the nucleus. The p65 subunit is then acetylated by p300/CBP or PCAF at K218, K221, and K310 to enhance NF- $\kappa$ B binding with DNA and promote its transcriptional activation ability. Moreover, deacetylation by HDAC3 promotes NF- $\kappa$ B nuclear export through binding with its repressor.<sup>103</sup> Therefore, the antitumor effect of HDACs inhibitors also partially depends on the deacetylation of NF- $\kappa$ B to promote transcription of apoptosis genes.

The signal transducers and activators of transcription (STAT) protein family regulate cell survival, growth, and differentiation. Dysregulation of STATs is frequently observed in primary tumors and leads to increased tumor growth and angiogenesis. Acetylation at K685 by CBP/p300 modulates the dimerization of STAT3, which enhances DNA binding and gene transcription (such as *cyclin D1*, *Bcl-xL*), and HDAC3 is primarily responsible for its deacetylation.<sup>104</sup> In melanoma cells, CBP acetylates STAT1 at K410 and K413 and induces cell apoptosis.<sup>105</sup>

Acetylated STAT1 also preferentially binds to p65 and enhances the transcription of NF- $\kappa$ B target genes.

The Wnt signaling pathway has a crucial role during embryonic development, whereas its dysregulation causes cellular transformation and a variety of human cancers. Lymphoid enhancer factor/T-cell factor (LEF/TCF) mediates Wnt signaling in the nucleus by recruiting  $\beta$ -catenin and its co-activators to target genes. In *C. elegans*, acetylation enhances the nuclear retention of POP-1, the LEF/TCF homolog, and activates its biological function during embryogenesis.<sup>106</sup> Another central component of the Wnt pathway,  $\beta$ -catenin, is also acetylated by CBP at K49, which is located in an important region that is frequently mutated in cancers. Mutation of K49 increases the ability of  $\beta$ -catenin to activate the *c-Myc* gene. Many investigations have indicated that Wnt signaling is also responsible for the metabolic programming of glycolysis in some cancers.<sup>107</sup> These results suggest that acetylation of the Wnt/ $\beta$ -catenin pathway is involved in tumor metabolism.<sup>108</sup>

Alteration of EGFR is involved in many cancers and has been intensively investigated. There is evidence that acetylation of EGFR plays a pivotal role in controlling its function and metabolism. EGFR can be acetylated by CBP. This acetylation influences the tyrosine phosphorylation of EGFR, which may contribute to resistance of cancer cells to HDAC inhibitors. This study indicated that a combination of HDAC inhibitors and EGFR inhibitors enhances therapeutic outcomes.<sup>109</sup>

So far, many investigations have shown that acetylation plays an important role in the development and progression of human

cancers, and several HDAC inhibitors such as SAHA have been approved by the FDA for cancer therapy in clinical practice. Recent studies found that several SIRT family members act as both oncogenes and tumor suppressor genes in a cell context-dependent manner. The identification of an increasing number of acetylation-modified substrates suggests that the acetylation network responsible for the regulation of tumor metabolism is more complicated than previously thought. Efforts to understand how acetylation coordinately modulates different metabolic and related pathways globally and contributes to tumorigenesis and tumor development are ongoing, but it is hoped that the results of these studies will provide a number of potential targets for cancer therapy.

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